University of Alberta

CEREAL BETA-GLUCAN: STRUCTURE AND FUNCTION

by

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This thesis is dedicated to Puja and Ekam Ghotra
my Daddy Ji and Mummy Ji
&
also to the new baby who is eagerly awaited as an addition to our
Happy Family

It is their love, understanding, encouragement and care that gave me the strength to reach for high standard of educational achievements and to continue this in future endeavors....

Abstract

Structural and functional properties of β-glucan purified from a cereal β-glucan concentrate which is manufactured by a novel patented technique were investigated. The objective of this thesis is to investigate the fine structure and functional characteristics of cereal β-glucans while acknowledging the intimate relationship between structure and function from both rheological and physiological perspectives.

Nuclear magnetic resonance (NMR) spectroscopy revealed that the chemical structure of β-glucan purified from barley fiber concentrate was similar to that reported in the literature. Fine structural NMR investigations elucidated that β-glucan is associated with phosphate moieties in both oat and barley concentrates (degree of substitution (DS) = 0.011 and 0.005, respectively), thus documenting for the first time that β-glucan may be associated with a negative charge. The higher prevalence of negatively charged phosphate moieties in oat β-glucan compared to barley may help to explain the higher solubility of oat β-glucan (6.6-fold greater than barley β-glucan at 37°C).

Rheological properties such as flow behavior, viscosity, viscoelasticity and thixotropy of solutions of β-glucan purifed from barley fiber concentrate and 12 common food gums, alone and in combinations, were characterized using an oscillatory rheological measurements. Pure gums and gum combinations were evaluated at 0.5 and 0.75% (w/w) total gum concentration in aqueous medium, whereas the β-glucan-gum ratio was kept at 90/10 or 80/20 (w/w). Viscosity

synergism was observed for β-glucan solutions in combination with xanthan, *iota*-carageenan, and carboxymethyl cellulose. In addition, β-glucan/xanthan gum blends demonstrated improved shear tolerance, gelling tendency and improved phase stability and viscosity stability (pH 3.5).

To evaluate the ability of cereal β-glucan to lower serum cholesterol levels in the body, *in vitro* adsorption of bile acids was chosen as an indicator of physiological functionality. A new quantitative method for determining bile acid adsorption of soluble fibers was developed, which was simpler, faster, and could accommodate higher throughput than conventional methods. β-Glucan purified from the fiber concentrate adsorbed bile acids significantly ($p \leq 0.5$) better than any of the twelve gums studied. This body of work establishes the potential of the β-glucan concentrate to serve as a cholesterol-lowering food ingredient in addition to being an excellent thickener.

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Special thanks to Dr. Feral Temelli for her insights and useful suggestions that helped me in completing my research. Many thanks to Dr. Steve Cui, my external examiner, for coming here all the way from Ontario to sit on my defense committee. I would also like to thank Dr. David Bressler, Dr. Raimar Löbenberg, and Dr. Nat Kav for taking the time out of their busy schedule to be on my defense committee.

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LIST OF ABBREVIATIONS

ALG	Alginate
BBG	Barley β-glucan
CAR	Carageenan
CEL	Cellulose
CHO	Cholestyramine resin
CMC	Carboxymethyl cellulose
DPM	Disintegrations per minute
GAR	Gum arabic
Glc*p*	Glucopyranosyl unit
GUA	Guar gum
HMP	High methoxyl pectin
HPLC	High performance liquid chromatography
KOG	Konjac
LBG	Locust bean gum
LMP	Low methoxyl pectin
MALDI-TOF MS	Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry
MCC	Microcrystalline cellulose
NMR	Nuclear magnetic resonance
OBG	Oat β-glucan
RS	Resistant starch
SCFA	Short chain fatty acids
XAN	Xanthan

Chapter 1
INTRODUCTION AND OBJECTIVES

Over the past few years, value-added processing of agricultural commodities (such as barley, oat, rye, wheat, etc.,) has received increasing attention from the food industry and researchers in universities and government institutions. Value-added processing brings higher returns to the farmers and economy while filling the specialized market demand of functional foods. This thesis focuses on fundamental research on β-glucan, a high-value component extracted from barley and oats, and its utilization in foods.

β-Glucan, a soluble dietary fiber, is a major component of the cell walls of cereal grains such as barley and oat. β-Glucan exhibits two major health benefits that include lowering serum cholesterol and regulating blood glucose levels (Anderson and Chen, 1979; Jenkins et al., 1985; McIntosh et al., 1991; Newman et al., 1992; Eastwood, 1992; Newman and Newman, 1992; Anderson et al., 1992). Cholesterol lowering has been associated with the reduction of cardiovascular disease risks that have led to the allowance of a health claim for oat products in the USA (FDA, 1997). Similar health benefits of β-glucan in barley led to the submission of a petition by the National Barley Food Council (Washington D.C.) in 2004 for a health claim similar to the one allowed for oats. As a result, the Food and Drug Administration has approved a barley health claim recently (FDA, 2005).

In addition to its health benefits, β-glucan has some technological value as it also functions as a hydrocolloid. Similar to common food gums or hydrocolloids, β-glucan contributes to viscosity and gelling behavior in certain food systems. Due to

many of its physicochemical properties, β-glucan from both barley and oat has received worldwide attention from scientists and product manufacturers. In order to use cereal β-glucans in novel food applications, it is essential to elucidate fundamental structural and functional properties of β-glucans (characterizing both physical as well as physiological functionality). The overall objective of the present thesis is to evaluate fine structure and functional characteristics of β-glucan purified from a cereal β-glucan and elucidating the structure-function relationships β-glucans from both rheological and physiological functionality. The main objectives of this research are as follows:

1. to determine the fine structure of barley β-glucan in a concentrate that is produced at a commercial scale using a novel patented technology, and to compare its structure with the documented β-glucan structure reported in literature (Chapter 3),
2. to determine the phosphorous content of barley and oat β-glucan concentrate and the characterization of phosphorous in terms of its form (i.e. free or bound) and location in the molecule (Chapter 4),
3. to evaluate the rheological properties of pure β-glucan dispersions and its blends with commercial food gums (Chapter 5), and
4. to develop a rapid methodology in order to quantitatively determine and compare the *in vitro* bile salt adsorption efficacy of barley β-glucan with that of commercial gums (Chapter 6).

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Chapter 2
LITERATURE REVIEW

2.1 Introduction

Cereal grains are produced in greater quantities worldwide than any other crop and used as food for humans and feed for livestock. Cultivation of wheat and barley has been reported as early as 11–12 thousand years ago (Hillman et al., 2001), whereas oat (*Avena sativa* L.) was domesticated for human consumption much later (1000 A.D.).

Total worldwide production of all cereal grains is 2,264 million MT per annum (FAOSTAT, 2005). Of these, corn, wheat and rice are the leading crops produced worldwide, accounting for 32%, 28%, and 27%, respectively, of the total world production. Barley is the next most abundant crop accounting for 7% of worldwide production, and oat accounts for approximately 1% (FAOSTAT, 2005). The leading barley producer is the European Union (51.6 million MT) followed by the Russian Federation (25.0 million MT), and Canada (13.1 million MT) (Brennan and Cleary, 2005).

In Canada, the average annual cereal harvest of 52.6 million MT is predominated by wheat (25.8 million MT), barley (13.1 million MT) and oat (3.6 million MT) (Canadian Wheat Board, 1998). Most of this grain is produced in the Prairie Provinces, Manitoba, Saskatchewan, and Alberta. Alberta remains the highest barley producer, growing approximately 50% of all barley produced in Canada. The average Canadian production of barley for the past 10 years remained between 12-13 million MT per year (Canadian Wheat Board, 1998).

2.1.1 Utilization

With regard to human consumption, both oat and barley grains were previously considered to be under-utilized. Widespread cultivation of oat originally occurred upon recognition of its nutritional properties. The three distinct end uses of barley are malting and brewing for the manufacture of alcoholic beverages, human food and livestock feed. In Western countries, 80 to 90% of barley production is for malt and animal feed (Jadhav et al., 1998). The lower end value uses (i.e., livestock feed) brings relatively low returns to the farmers and economy. In Canada, only 5% of the barley produced is utilized for human consumption (non-alcoholic food use). It is consumed mainly in the form of pot and pearled barley in breakfast cereals, soups, porridge, and baby foods (Bhatty, 1986 and 1993). For oats, 78% of the world production is used for livestock feed, 18% for human food, and the remaining 4% for other purposes (Schrickel, 1986).

Much research on barley has focused on its malting and brewing potential (Bathgate et al., 1974; Bamforth et al., 1979; Henry and Blakeney, 1986; Palmer, 1987; Brennan et al., 1996, 1997; Molina-Cano et al., 2002; Edney and Mather, 2004). However, in the past two decades, a number of compositional and nutritional studies on barley and oat grains have demonstrated their superior nutritional quality for human consumption. Thus, increasing recognition of the nutritional value coupled with increased health consciousness of consumers has resulted in increased demand for oat and barley grains. Much attention has been given to the potential higher end utilization of these grains by isolating certain value-added components,

such as β-glucan. β-Glucan is considered to be an important nutraceutical for functional foods (Malkki, 2004; Trepel, 2004) that exert beneficial effects and/or reduce the risk of chronic diseases (Anderson and Chen, 1979; Vahouny et al., 1980; Jenkins et al., 1985; Anderson et al., 1992; Malkki 2004; Behall and Hallfrisch, 2006).

Recently, researchers and industry have shown increased interest towards developing new β-glucan isolation/concentration technologies. This enables value-added utilization of barley and oat grains for non-traditional food uses. Table 2-1 presents recently developed commercial activities targeting β-glucan concentrates, worldwide, showing value added utilization of oat and barley.

2.2 Structure of oat and barley grains

Cereal plants produce one seeded dry fruit called a caryopsis which is commonly called grain or groat (i.e. grain devoid of hull). The schematic of the anatomical structure of a typical barley and oat grain is presented, respectively in Figures 2-1a and 2-1b. The outermost part of the grain is called the husk/hull, which adheres to the developing seed except in hull-less varieties. The husk/hull, composed of lemma and palea layers, comprises on average about 23% of the weight of the whole grain (Magness et al., 1971). In barley and oats, the specific values are approximately 25% and 35% (w/w), respectively (personal communication with Dr. Rossnagel). The husk/hull is highly fibrous in nature and is primarily composed of

Table 2-1 Recently developed commercial activities worldwide (since 2000) showing value added utilization of oat and barley

Company	Technology	Target cereals	β-Glucan purity, w/w
Cevena Bioproducts Inc. (Canada)	Concentration of β-glucan using aqueous-alcohol and enzymatic process and ring drier recovery	Oat/barley	Oat- 50% Barley – 60%
GraceLinc Ltd. (New Zealand)	Extraction of β-glucan using aqueous-alkali solubilization and freeze thaw recovery	Barley	75-80%
Nurture Inc. (USA)	Extraction of β-glucan using aqueous-alkali solubilization and recovery by drum drying	Oat	54%

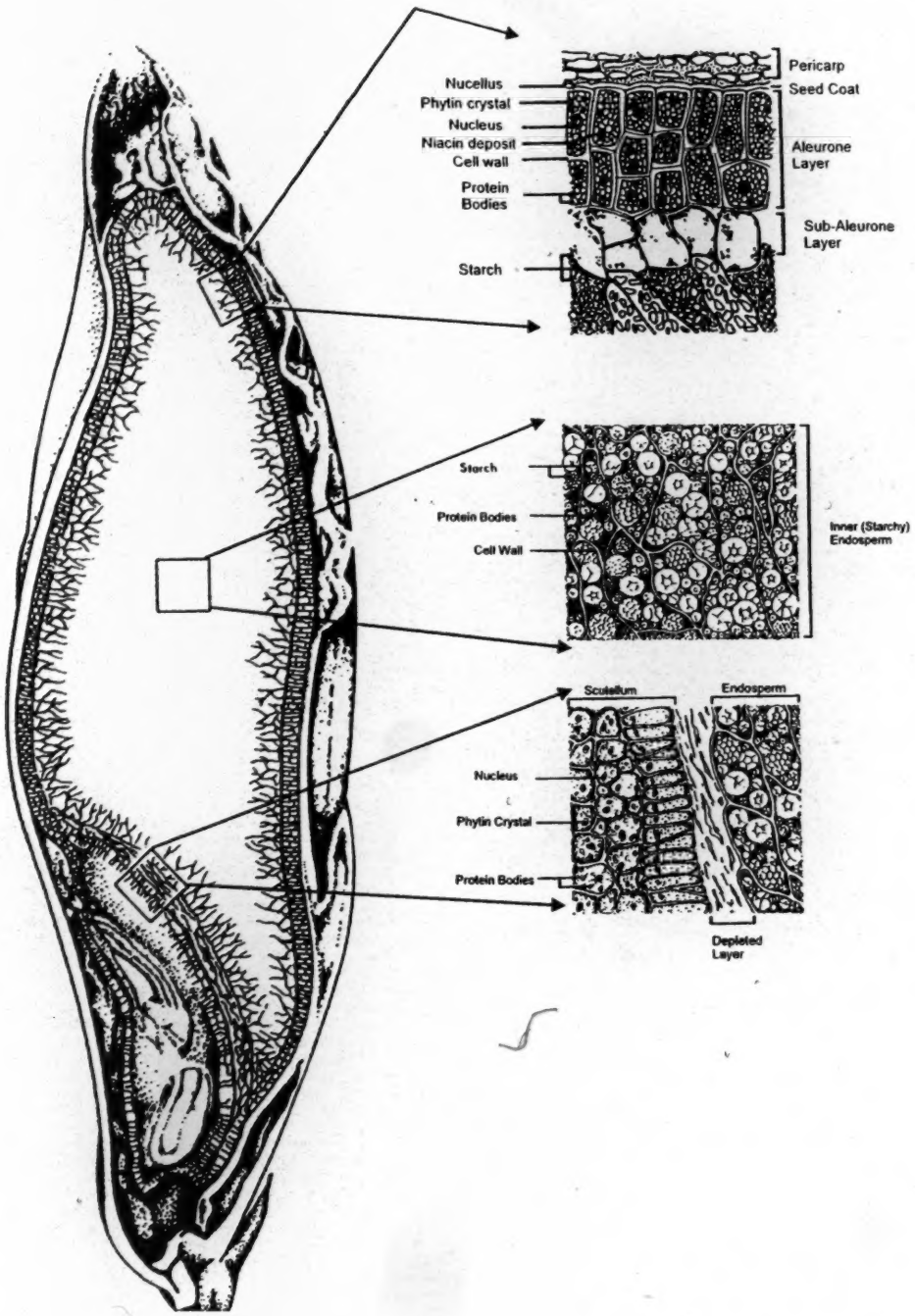


Figure 2-1 (a) Schematic of the anatomical structure of a typical barley grain (Adapter with minor modification from Newman and Newman, 1991)

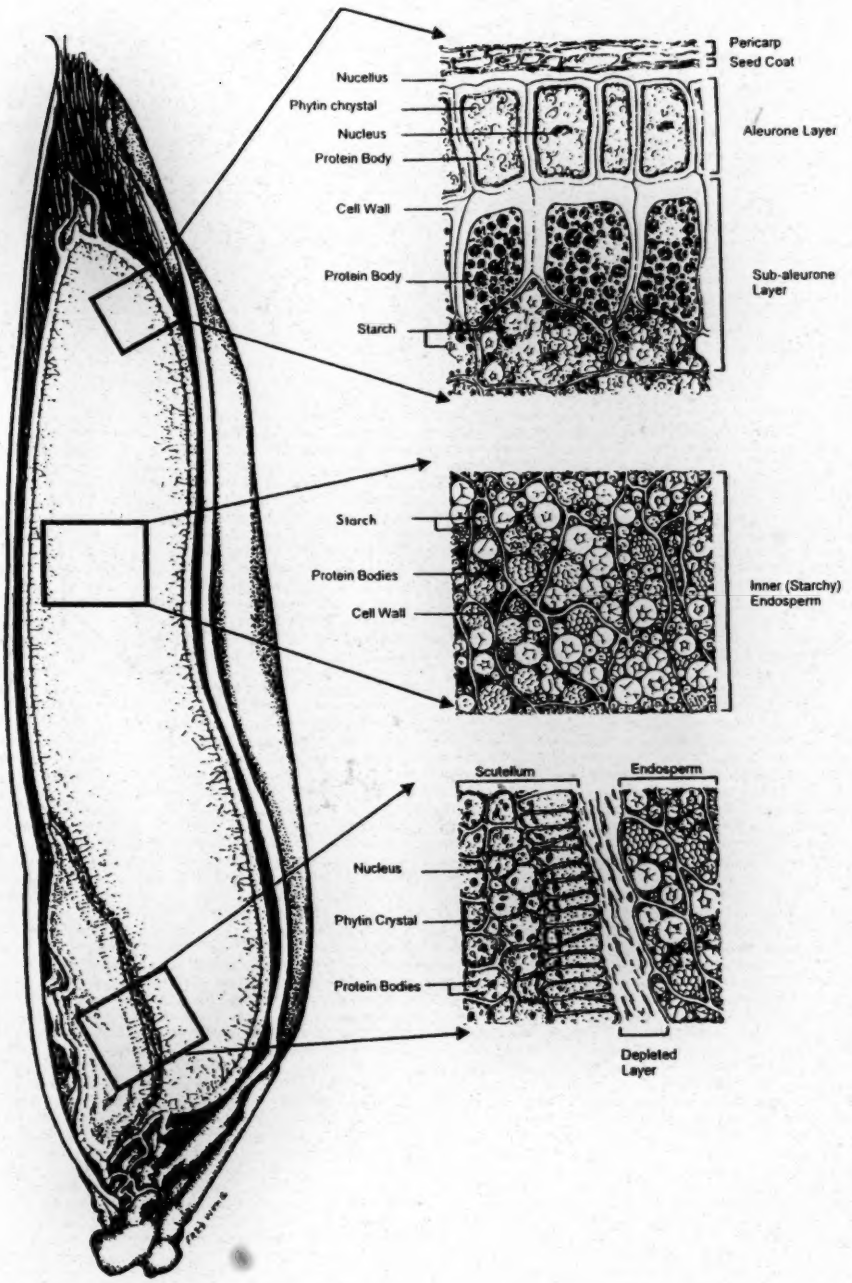


Figure 2-1 (b) Schematic of the anatomical structure of a typical oat grain (Adapted with minot modification from Fulcher and Miller, 1993)

cellulose and hemicellulose, but also contains significant amounts of lignin. Below the husk is the fruit coat (also known as the pericarp), which is composed of hemicellulose and cellulose but lacks lignin. The pericarp, seed coat, aleurone and sub-aleurone layers are collectively called bran. The pericarp is attached to the testa, which surrounds the endosperm. The endosperm is the major storage organ for starch and protein.

Starch in the endosperm is present in a pebble-like form embedded in the protein matrix. The bran, in both oats and barley, are rich in phenolic pigments. The aleurone layer surrounds the endosperm. The cell walls of the endosperm and aleurone layers consist mainly of mixed linkage β-glucan, arabinoxylans, and small amounts of protein. Of all cereals, the largest quantities of β-glucan are found in barley and oats with levels ranging from 3-11% and 3-7%, respectively (Bhatty 1992; Marlett, 1993; Burkus and Temelli, 2000; Skendi et al., 2003). The germ of both oat and barley consists of the scutellum and embryo, which are rich in lipids and protein.

2.3 Chemical composition of barley and oat grains

Barley (*Hordeum vulgare* L.) and oat (*Avena sativa L.*) grains, like most other cereal grains, contain carbohydrates (starch and non-starch polysaccharides, oligosaccharides and sugars), proteins, lipids, minerals, vitamins and other minor components. The chemical composition of grains is dependent on many factors such as genotype and environmental conditions including temperature, day length, water

supply, and the availability of soil minerals (Yoon et al., 1995; Zhang et al., 2002). The average chemical composition of barley and oat grains is summarized in Table 2-2. In general, the chemical components are not uniformly distributed in the grain. For example, hull and bran are rich in cellulose, pentosan and ash whereas, the endosperm is rich in starch and protein. The cell walls of barley bran usually contain relatively high levels of pentosan but low levels of β-glucan as compared to the cell walls of endosperm (Henry, 1986). Furthermore, in oat, the β-glucan is more concentrated in the outer bran layers as compared to that of endosperm (Marlett, 1993; Yokoyama et al., 2002). Also, aleurone layers of oat and barley have been shown to contain more minerals and proteins as compared to the endosperm. Lipids are generally concentrated in the bran and germ.

2.4 Starch

Starch, the major energy reserve, is a polysaccharide present in the endosperm part of cereal grains. In cereals, it occurs in the form of discrete granules of various shapes and sizes. The starch granule is a homo-polysaccharide composed of variable proportions of linear α-(1→4)-glucan, called amylose, and the branched amylopectin, where linear α-(1→4)-linked-glucan chains are connected through α-(1→6) linkages (MacGregor and Fincher, 1993). Amylose has a molecular mass in the range of 10^5 to 10^6 Daltons, and the linear α-(1→4) linked glucan chains form a spiral shape (single or double helix). In contrast, amylopectin is one of the largest polysaccharides known with molecular mass in the range of 10^7 to 10^9 Daltons and the α-(1→4) linked glucan chain is involved in extensive multiple branching by

Table 2-2 Typical chemical composition of barley and oat grains

Component	(%, w/w dry weight)	
	Barley[a]	Oat[b]
Starch	60–64	55.8-63.6
Proteins	8–15	11-24
Lipids	2–3	6.6-8.8
β-Glucan	3.6–6.1	4.8-6.6
Total dietary fiber	1.4–5.0	7.1-12.1
Simple free sugars	0.41–2.9	2.3
Minerals (ash)	2–3	1.7-2.9

[a] Source: MacGregor (1993).
[b] Source: Marlett (1993)

α-(1→6) linkages (Zobel, 1988; Manners, 1989). The most investigated physicochemical properties of starch are its gelatinization and melting behavior, especially during food processing. Some obvious examples are the viscosity and mouthfeel of gravies and puddings and the texture of gum drops and pie filling. Starch, although generally considered to be fully digestible, also contains a fraction, which is partly indigestible in the intestinal tract of humans. This fraction of starch resisting digestion in the human intestine is known as resistant starch (RS). Due to the excellent fermentative capacity of the gut, particularly regarding its yield of butyric acid, resistant starch is considered a new tool for the formulation of fiber-rich foods.

2.5 Non-starch polysaccharides

Non-starch polysaccharides (NSPs) belong to a class of carbohydrate known as dietary fibre (DF) which is defined as the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine (AACC Report, 2001; AACC, 2003). Dietary fibre (DF) is often divided into water-soluble and water-insoluble fractions. Cellulose is the most common insoluble dietary fiber whereas β-glucan and arabinoxylans distribute into both soluble and insoluble fractions.

Cellulose is the major non-starch polysaccharide (NSP) that supports the structure of plants and it is the major constituent in the husk and outer bran layers of cereals. Cellulose consists of linear (1→4) linked β-glycosidic residues and

therefore has a tendency to tightly pack together into microcrystalline regions (Fincher and Stone, 1986). Two other major NSPs are arabinoxylans and β-glucans. Arabinoxylans, together with other NSPs such as β-glucans, form the cell walls of endosperm (Fincher and Stone, 1986; Muralikrishna and Tharanathan, 1986). Arabinoxylans are composed of (1→4)-linked β-D-xylopyranosyl residues, which are substituted at either 3- or both 2– and 3-positions with α-L-arabinofuranosyl residues (Tharanathan, 2002). Oligomeric sequences consisting of either arabinose or arabinose and xylose have been identified in oat and barley (Tharanathan, 2002; Virkki et al., 2005). The ratio of arabinose and xylose (Ara/Xyl) indicates the degree of branching in arabinoxylan polysaccharides. The higher the ratio, the higher the degree of branching. The average Ara/Xyl ratio in both oats and barley has been reported to be 0.50 by Virkki et al. (2005). Branching appears to strongly affect the solubility of arabinoxylans. Arabinoxylans with an intermediate Ara/Xyl ratio have relatively high solubility, whereas arabinoxylans with low or high Ara/Xyl ratios generally exhibit lower solubility (Virkki et al., 2005).

2.6 β-Glucan

β-Glucan is a cell wall component of cereal grains that exists in appreciable amounts in barley (3-11%) and oats (3-7%), while in wheat and rye it is present in lesser quantities (0.5-1% and 1-2%, respectively) (Bhatty 1992; Skendi et al., 2003). Cereal β-glucan is a linear polysaccharide, which is composed of β-(1→3) and β-(1→4) linked glucopyranosyl residues. The structure features successively β-(1→4)-linked glucopyranosyl units (primarily cellotriosyl and cellotetraosyl), which are

separated by single β-(1→3) linkages as shown in Figure 2-2 (Igarashi and Sakurai, 1966; Woodward et al., 1983; Wood et al., 1991b;Wood et al., 1994; Cui et al., 2000). Linear β-(1→3) linkages occur singly and β-(1→4) linkages generally occur in sequences of 2 or 3, but consecutive sequences of 4 to 14 β-glucosyl units has been reported in cereal β-glucans (Cui, 2001; Lazaridou et al., 2003). It is these (1→3) linkages that confer β-glucan with solubility by allowing a kink in the structure thereby preventing β-glucan strands from packing into cellulose-like regions which are insoluble in nature. As a result, water molecules are able to interact with β-glucan by forming hydrogen bonds with the hydroxyl groups of β-glucosyl subunits (Macgregor and Fincher, 1993).

The ratio of cellotriosyl to cellotetraosyl residues (represented by DP3 and DP4 oligomers that are released by β-glucan hydrolysis by lichenase) of oat and barley β-glucan is presented in Table 2-3. Barley has a higher ratio of cellotriosyl to cellotetraosyl residues than oats (Lazaridou et al., 2003). The ratio of cellotriosyl to cellotetraosyl residues of β-glucan is important in terms of the solubility and rheological properties of aqueous dispersions of β-glucans (Wood et al., 1991a; Wood et al., 1994; Cui, et al., 2000).

A wide range of molecular weight values from 20,000 to 3,000,000 Daltons are reported for oat and barley β-glucans (Wood et al., 1991b; Cui, 2001; Yokoyama et al., 2002; Lazaridou et al., 2003). The variation in molecular weight of cereal β-glucans may be attributed to type of cultivar and the environment where the crop is grown. Extraction techniques for the isolation of β-glucan (effect of solvent, pH,

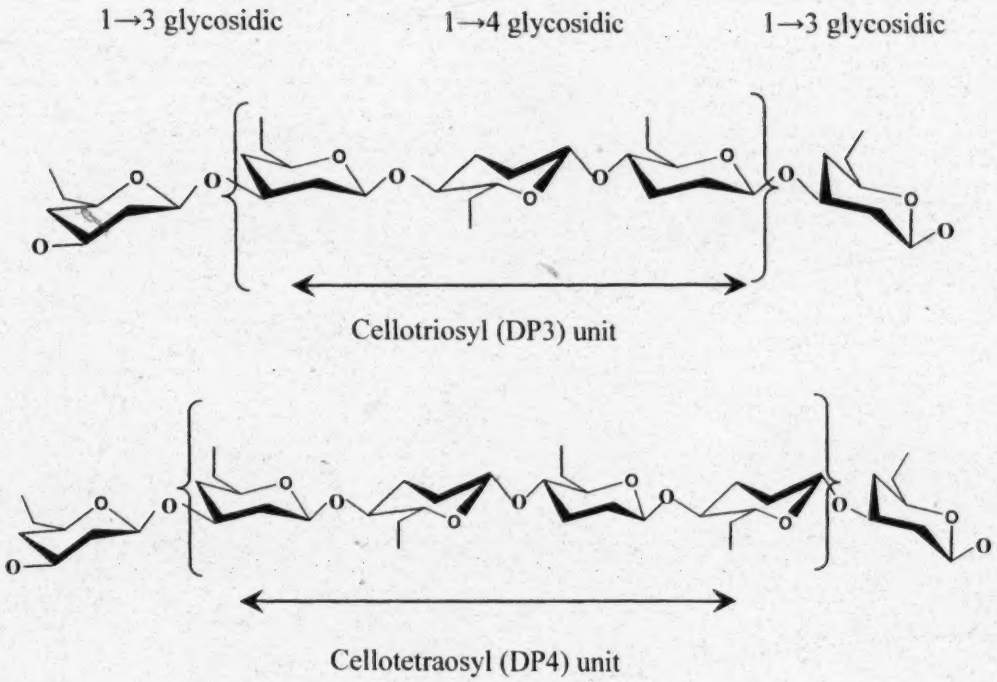


Figure 2-2 Molecular structure of β-glucan

Table 2-3 The ratio of cellotriosyl-to-cellotetraosyl residues of oat and barley β-glucans

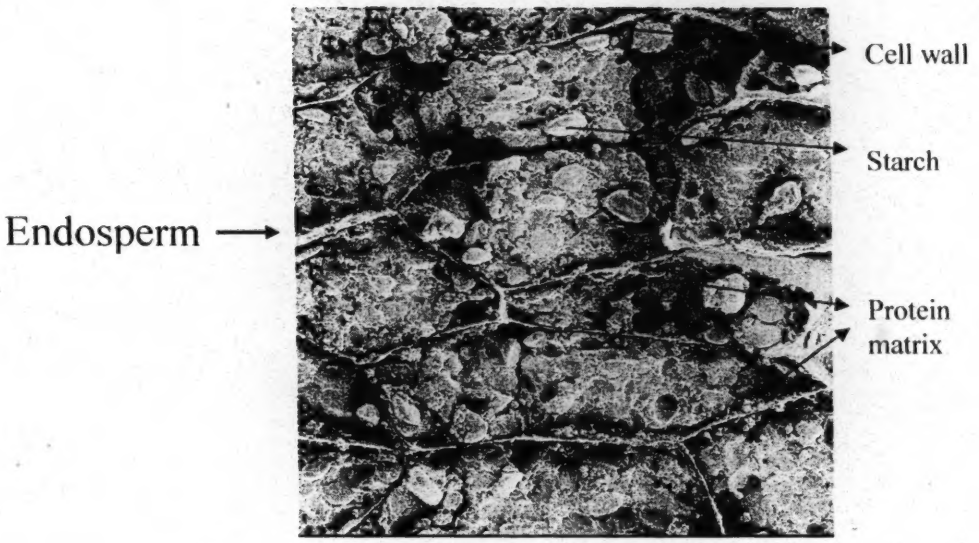
Source	DP3: DP4
Barley β-glucan	2.3-2.6[a]
Oat β-glucan	1.8 – 1.2[b]

[a] Jiang and Vasanthan (2000)
[b] Wood (1993); Wood et al. (1989)

temperature, presence of endogenous enzymes, and shear during processing) also plays a major role in the resulting molecular weight of isolated β-glucan (Izydorczyk and Biliaderis, 2000). The molecular weight of β-glucan affects its solution properties in terms of viscosity, gelling behavior, and other rheological properties.

2.7 β-glucan isolation/concentration technologies

The techniques to concentrate β-glucan include dry milling and sieving or air-classification (Wu et al., 1994; Knuckles and Chiu, 1995; Vasanthan and Bhatty, 1995; Bhatty, 1997) and wet techniques such as aqueous/aqueous-alkali and alcohol-based enzymatic techniques (Burrows et al., 1984; Myllymaki et al., 1989; Wood et al., 1989; Inglet 1992; Burkus and Temelli, 1998; Vasanthan and Temelli, 2002). Dry-milled flour is used as the feed stock for dry and wet concentration techniques. The flour is comprised of a mixture of particulates that are simple and complex in nature as shown in Figure 2-3. These particulates vary in their physical properties such as size, shape and density. Flour fractions containing β-glucan up to ~7-8% can be prepared by simple vibratory sieving that fractionates particulates based on their size. However, air-classification of the flour separates and concentrates β-glucan rich particulates primarily based on their shape and density. A fiber concentrate containing up to ~20-24% β-glucan (Vasanthan and Bhatty, 1996) may be obtained through optimization of air-classification parameters such as flour feed rate, air flow rate, classifier wheel speed, etc. A relatively low β-glucan concentration of the fiber concentrates produced through dry techniques present challenges in food formulations targeting to meet the FDA health claim at 0.75 g β-glucan per serving.



Dry Milling

Complex nature of particulates in dry milled flour such as:

- Starch-protein complex
- Starch-protein-fiber complex
- Starch-fiber complex
- Protein-fiber complex
- Starch alone
- Protein alone
- Fiber alone

Figure 2-3 Particulate nature of dry milled flour

Unlike dry separation techniques, the wet techniques can yield fiber concentrates with a high β-glucan concentration (>50%, w/w). The aqueous or aqueous-alkali techniques involve four major steps (Figure 2-4): a) solubilization of β-glucan from flour or bran in aqueous or aqueous-alkali, b) centrifugation of the slurry to separate the solid phase from the liquid phase containing solubilized β-glucan, c) acid and/or heat precipitation and centrifugal removal of proteins from the liquid phase, and d) recovering β-glucan from the liquid phase by alcohol precipitation or by simple drum/tray drying. Proprietary variations in the aforementioned steps have been employed by different producers/researchers (Potter et al., 2003; Morgan, 2003)

β-Glucan concentrates produced commercially through aqueous technologies, show low solution viscosity upon reconstitution in water. This is due to the fact that in the aqueous process, β-glucan is solubilized from the cell wall and in the process, endogenous enzymes such as cellulase and β-glucanase that are native to flour hydrolyse the β-glucan molecule thereby decreasing its molecular weight. Also, in an aqueous solution, the hydrated β-glucan molecules become highly susceptible to shear fragmentation during mixing and centrifugation steps of processing. Another disadvantage of the aqueous technologies is the use of substantially large quantities of water, which needs to be evaporated to dry the fiber concentrate. This significantly increases the cost of production. β-Glucan concentrations up to 90% can be achieved by aqueous or aqueous-alkali technologies. Another aqueous technology (Inglett, 1992) for β-glucan concentration uses an approach that solubilizes β-glucan and starch with the application of heat in the presence of

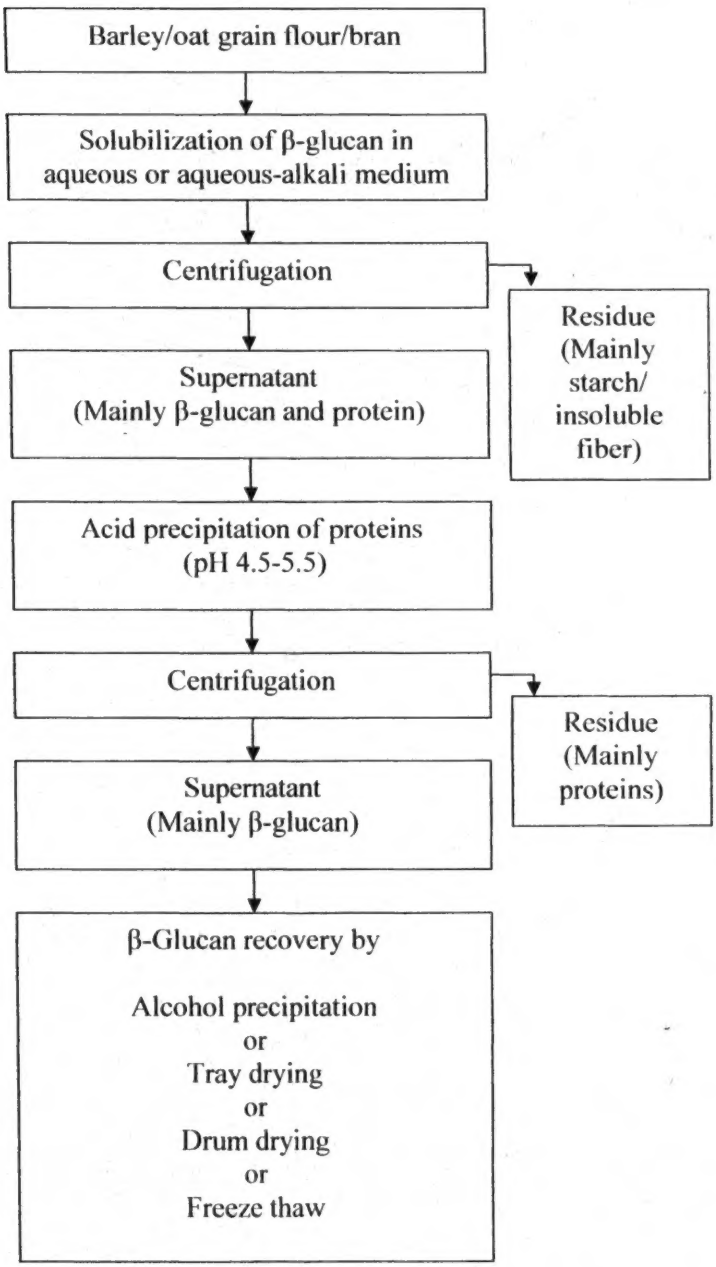


Figure 2-4 A flowchart illustrating the primary steps in the extraction of β-glucan using aqueous or aqueous-alkali solubilization

thermostable alpha-amylase. The liquid phase containing the solubilized β-glucan and hydrolyzed starch is separated by centrifugation and dried to obtain a powder. β-glucan concentration upto 10% can be achieved by this process.

Recently, a cost effective alcohol-based enzymatic technique to manufacture β-glucan fiber concentrates was introduced (Vasanthan and Temelli, 2002) (Figure 2-5). In this technology, grain flour is slurried in aqueous ethanol and treated with specialty enzymes to hydrolyze protein and starch. Subsequently, the β-glucan enriched fiber particulates are recovered by simple filtration techniques. In the presence of alcohol, β-glucan is not solubilized and remains intact within the cell wall. This technology has been shown to yield fiber concentrates with superior β-glucan characteristics (i.e. high molecular weight and viscosity) as compared to those produced through aqueous/aqueous-alkali technologies. Since β-glucan is not solubilized from the cell walls, potential hydrolysis by endogenous enzymes such as cellulase and β-glucanase is minimized. Also, shear fragmentation of the molecule does not occur as β-glucan remains unsolubilized throughout the process, preserving the original molecular weight and viscosity characteristics. β-Gucan concentrations of ~50% (w/w) and ~60% (w/w) have been obtained for oat and barley β-glucan concentrates, respectively, by this technology.

2.8 Rheological properties of β-glucan

Similar to other polysaccharides (e.g. guar, xanthan, locust bean gum etc.), solutions of cereal β-glucans fall into the category of pseudoplastic and viscoelastic fluids (Lazaridou et al., 2003). The rheological properties of β-glucan are dependent

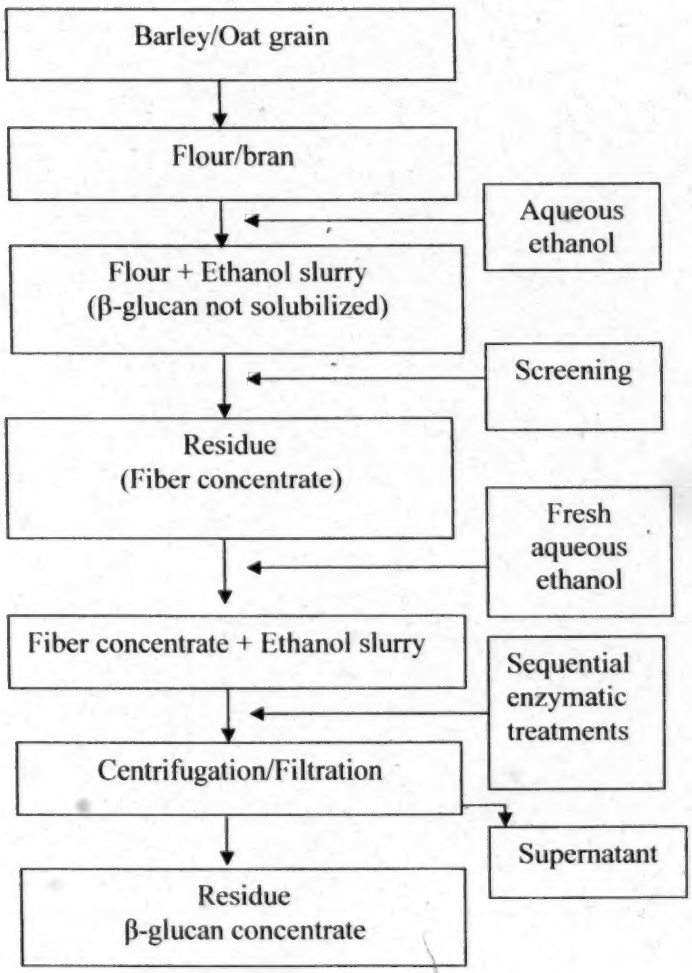


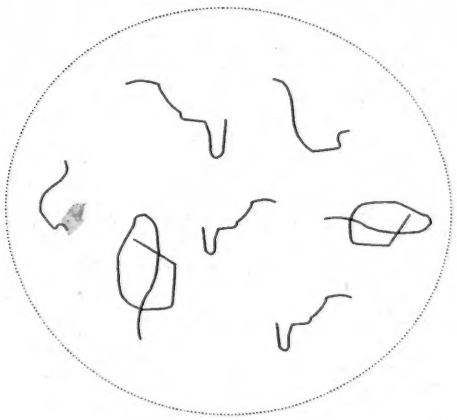
Figure 2-5 Concentration of β-glucan using aqueous-alcohol and enzymatic process

upon polysaccharide concentration, processing history and molecular properties such as molecular weight and primary structural features (i.e. ratio between 1→3 and 1→4 linkages).

Cereal β-glucan, at relatively low concentrations, exists as random coil in solution. At a critical concentration, individual β-glucan strands can self-associate or can associate with other β-glucan strands to form junction zones. Junction zones are formed by hydrogen bonding between linear cellulosic regions (Figure 2-6). The junction zone formation above a critical concentration creates a micelle structure (Grim et al., 1995). A typical micellar structure is shown in Figure 2-6 (C). The mechanism behind micelle formation is likely intra- and intermolecular strong non-covalent interactions (e.g. formation of hydrogen bonding as shown in Figure 2-7) between regular cellulosic regions of β-glucan chains (Woodward et al., 1983; Doublier and Wood, 1995; Gomez et al., 1997a; Bohm and Kulicke, 1999). However, another mechanism suggested was aggregate formation through the junctions between consecutively occurring cellotriosyl units (Bohm and Kulicke, 1999; Cui et al., 2000). In viscous solution at critical concentration the micelles are well separated, however inter-micelles association (by hydrogen bonding) results in three dimensional network formations, which are characteristic of gels.

Rheological properties such as flow behavior, viscosity, viscoelasticity and thixotropy of polysaccharides, including cereal β-glucan, have been studied previously (Skendi et al., 2003; Lazaridou et al., 2003). Cereal β-glucan solutions typically exhibit non-Newtonian flow behavior called shear thinning or pseudoplastic

(A) Random coil:
β-glucan, at low concentration, exists in solution as random coil



(B) Junction zone formation:
Schematic of a hydrogen bond formation resulting in junction zone formation involving two strands or one strand at critical concentration of β-glucan in solution

(C) Spherical micelles formation:
Schematic of spherical micelle formation at critical concentration of β-glucan in solution. For relatively low polymer concentration the micelles are well separated

Figure 2-6 Schematic of junction zone formation and micellar network formation

(D) Micelle Network formation:
Schematic of micelle network formation at critical concentration of β-glucan in solution. Individual micelles-like structures connect to eachother thourgh intermolecular forces

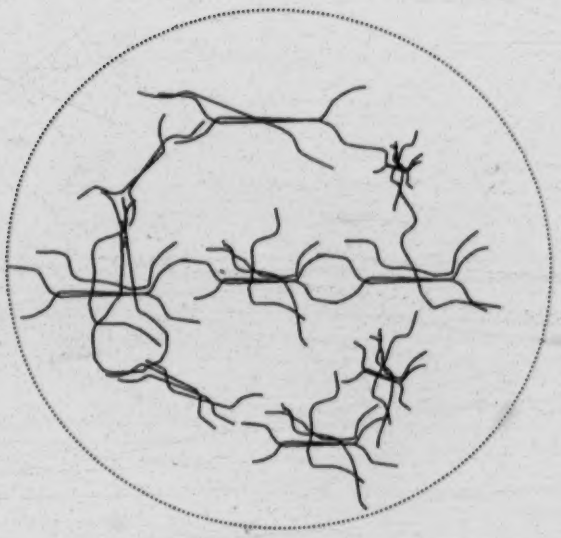


Figure 2-6 (Continued)

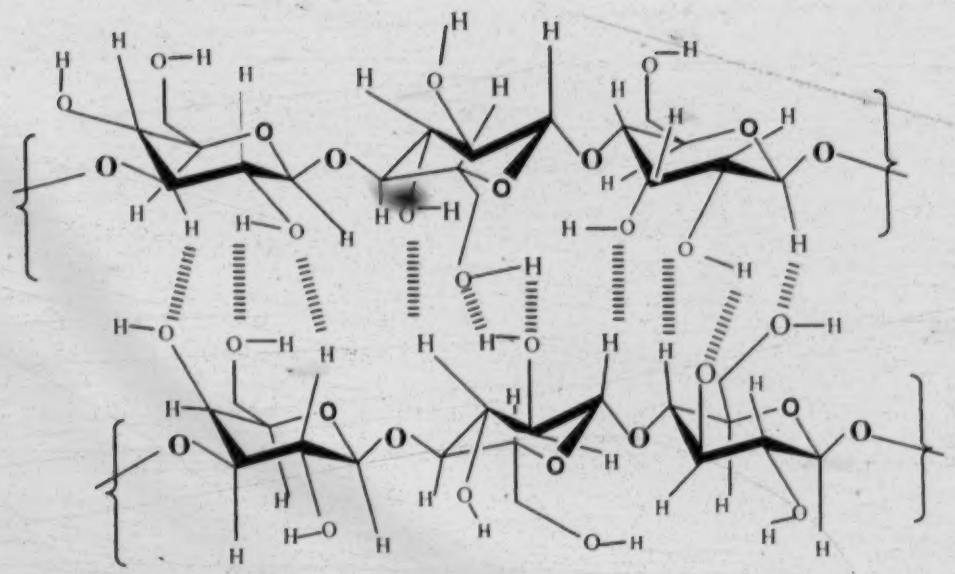


Figure 2-7 Schematic showing hydrogen bonding between hydroxyl groups of cellulosic backbone of β-glucan

flow behavior (Doublier and Wood, 1995; Lazaridou et al., 2000). In fluid flow behavior studies, the Power law model describes the pseudoplastic behavior of gums (Marcotte et al., 2001). The following equation represents the Power law model:

$$\tau = c\,\gamma^{n} \qquad (2\text{-}1)$$

where τ is the shear stress (N/m^2), γ is the shear rate (s^{-1}), c is the consistency coefficient and n is the flow behavior index or Power law index. Gum dispersions with a value of $n > 0.99$ are considered to be "Newtonian" whereas gums forming highly viscous solutions ($n < 1$) are termed pseudoplastic liquids (Marcotte et al., 2001).

Viscosity, measured in mPa.s, is a common way of characterizing liquids. Viscosity is resistance to flow when subjected to a force (called stress (N/m^2)). It is also described as the ratio of shear stress to shear rate.

Viscosity = Shear Stress/ Shear rate (2-2)

It is believed that the viscosity of neutral polysaccharide solutions (e.g. β-glucan), would not be affected by the pH of the aqueous medium. For ionic polysaccharides (e.g. pectin), pH plays a substantial role on viscosity characteristics (Whistler and BeMiller, 1999).

Viscoelastic properties of polymer gels are characterized by elastic modulus (G′) (or storage modulus) and viscous modulus (G″) (or loss modulus), respectively. When gum solutions are subjected to controlled strain (at constant frequency of 1 Hz) they exhibit a linear viscoelastic region. Deviations from linearity occur when the gel is strained to a point at which certain weak physical bonds of the

aggregated network structure are destroyed. Formation of new bonds will also influence the linear viscoelastic region. Freshly prepared barley β-glucan (BBG) dispersions have been reported to behave like a viscoelastic liquid (G″ > G′) where the G′ and G″ are reported to be highly dependent on frequency (Skendi et al., 2003). Frequency sweep is important to determine the time required for polymer entanglements to form or break within the variable periods of oscillations (Lazaridou et al., 2003). Formation of an elastic gel-like network (G′ > G″) depends on the gum concentration as well as the induction time of gelation. The induction time, i.e. the time required for the interactions to form a 3-D network, is a critical factor that controls the stability of the 3-D network. Once the gel-like viscoleastic properties are gained, the G′ and G″ become less dependent on frequency (Lazaridou et al., 2003). The gelling ability of cereal β-glucans follow the order of wheat> barley >oat; this trend seems to correspond with the ratio of cellotriosyl to cellotetraosyl units in the cereal β-glucan structure (4, 3 and 2 for wheat, barley and oat β-glucans, respectively). In addition to the structural features of cereal β-glucan, the gelation properties were also dependent on molecular size, concentration and gel curing temperature and time (Bohm and Kulicke, 1999; Cui and Wood, 2000; Lazaridou et al., 2003).

Thixotropy can be defined as a loss of viscosity due to destruction of the micellar structure under a constant shear rate over time or a consecutively increasing shear rate that is fixed for a period of time at each selected shear rate followed by redevelopment of the structure when shear is withdrawn (Muller, 1973; Schramm,

1994). The viscosity of non-thixotropic systems does not change under the application of fixed shear rates and their withdrawal.

2.8.1 Polysaccharide-polysaccharide interactions

Hydrocolloids or food gums are water-loving polysaccharides that have potential to function as thickeners and extenders in foods. In "hydrocolloid", the prefix "*hydro*" is the Greek word for water. The word colloid is derived from the French word "*col*" meaning glue and "*oid*" meaning like (William, 1977). Colloids form viscous sols at low concentration and gels at high concentration. Most of the hydrocolloids used in the food industry are derived from plants and marine algae (William, 1977).

Table 2-4 summarizes the origin of common food gums that are widely used in the food industry. The structure of various gums and their properties are summarized in detail by Glicksman (1969). All of these polysaccharides are hydrophilic and range from neutral to anionic in nature. In addition to their ionic nature, the structural diversity (e.g. linear, branched, etc.) of these polysaccharides is a major factor that affects their rheological properties in aqueous medium.

The spatial arrangement of these macromolecule mixtures in common foods, confers a specific structure and texture to a product. Binary blends of polysaccharides that are of industrial significance are listed in Table 2-5. Polysaccharide-polysaccharide mixtures often show better and more versatile functional properties than the individual components alone.

Table 2-4 Types of food gums

Source	Types of gums
Plant exudates[a]	Gum acacia, Gum tragacanth, Gum karaya
Seed gums[a]	Guar, Locust bean gum, Pectin
Seaweed extracts[a]	Carageenan, Alginate
Microbiologically synthesized products[b]	Xanthan gum
Chemically modified from cellulose[a]	Carboxymethylcellulose, hydroxypropyl cellulose
Chemically modified from starch[b]	Starch succinates, starch phosphates, hydroxypropylstarches
Synthetic[b]	Polydextrose

[a] Muller, 1973
[b] Whistler and BeMiller, 1990

Table 2-5 Common binary blends of polysaccharide showing viscous synergism

Viscosity enhancing combinations	**Reference**
Xanthan and starch	Mandala and Palogou (2003)
Xanthan and guar	Casa et al (2000)
Carboxymethyl cellulose and guar	Plutchok and Kokini (1986)
Starch and guar	Adamu and Yu (2002)
Carageenan and locust bean gum	Hernandez et al (2001)
Gelling combinations	
Xanthan and locust bean gum	Casa et al (1999); Whistler and BeMiller (1990)
Locust bean gum and *kappa*-carageenan	Tako et al (1998) Whistler and BeMiller(1990)

The food industry is continuously searching for economical ways of generating products with better desirable organoleptic properties (i.e. texture, mouthfeel, etc) than are currently provided by available hydrocolloids. Thus, despite the diversity of hydrocolloid functionalities at hand, researchers have demonstrated the use of existing hydrocolloids in innovative ways, such as blending two or more gums (Le Gloahec, 1951; Tako et al., 1998; Hernandez et al., 2001; Ikeda et al., 2004;). This approach offers opportunities for new product development. For instance, the addition of *kappa*-carageenan to locust bean gum produces highly stable thermo-reversible gels with important synergistic effects (Tako et al., 1998). A mixture of gum arabic and carrageenan as an ice cream stabilizer has been patented (Le Gloahee, 1951) and it functions to retard both ice crystal formation and growth. Hence, the establishment of fundamental rheological properties of gum blends and the understanding of the interactions of barley β-glucan with other food gums are of vital importance.

One of the major benefits of viscosity measurement is the ability to detect synergistic and antagonistic interactions in aqueous dispersions consisting of polysaccharide-polysaccharide mixtures (Nnanna and Dawkins, 1996; Pellicer et al., 2000; Hernandez et al., 2001). There are several definitions for synergistic and antagonistic interactions (Kalectunc-Gencer and Peleg, 1986; Plutchok and Kokini, 1986; Howell, 1994; Pellicer et al., 2000). In the present study, when the gum blend exhibits greater viscosity than the sum of the viscosities of the gum dispersions

considered separately, the situation was considered synergism. These interactions were quantified using a "viscous synergism index", I_v, that is defined as:

$$I_v = \frac{\eta_{i+j}}{\eta_i + \eta_j} \qquad (2\text{-}3)$$

where i and j represent the two gums forming the mixed system, $i + j$. The aqueous dispersions of the systems i, j and $i + j$ must be prepared at the same total gum concentrations, i.e., $c_i = c_j = c_{i+j}$ (Hernandez et al., 2001). According to Equation (2-3), I_v is always a positive value. If $0 < I_v < 0.5$, the viscosity of the mixed system will be less than the sum of the viscosities of its two component gums and also less than both of them individually, the situation is termed as antagonistic interaction. However, if $I_v = 0.5$ and both gums are of equal viscosity (when considered separately and at identical concentrations), so that $\eta_{i+j} = \eta_i = \eta_j$ then the situation is termed as no interaction. On the other hand, if $0.5 < I_v < 1$, synergism occurs, provided η_{i+j} is more than η_i and η_j individually. If $I_v > 1$, and if the viscosity of the mixed system is greater than the sum of the viscosities of the two individual systems i.e., $\eta_{i+j} > \eta_i + \eta_j$, then synergism has also occurred (Pellicer et al., 2000; Hernandez et al., 2001). For economical and practical reasons, blending of two pure gums together to increase the viscosity is not necessary when the viscosity of one of the pure gums, η_i or η_j, is $> \eta_{i+j}$ at identical gum concentrations (Hernandez et al., 2001). The synergistic effect on viscosity due to blending two polysaccharides is the most studied interaction. It has much significance to the industry and it is quite an inexpensive method of manipulating rheology and texture of food products.

Mixing two polysaccharides in an aqueous medium causes intermolecular interactions between 1) linear regions of the same polysaccharide; 2) linear regions of anionic polysaccharides as affected by the presence of divalent cations; 3) linear regions of two different polysaccharides. However, some other complex mechanisms of interactions are also suggested in the literature (Morris and Foster, 1994; Bresolin et al., 1998). As linear chain segments acquire more junction zones, at a critical level of intermolecular interaction, precipitation may occur. Therefore, to avoid precipitation, regular linear chain segments must be interrupted by irregularities so that interaction takes place over a limited region of the molecule so that the formation of junction zones is limited.

Depending upon the polysaccharide concentration used, intermolecular interactions between linear regions of polysaccharide molecules often result in the formation of a double helix. Further interactions between adjacent helical segments can occur to form a stable 3-D network, which leads to a gel structure. If junction zones grow after formation of a gel, the network becomes more compact, the structure contracts and syneresis occurs.

One way of extending the applications of soluble dietary fibers such as β-glucan, is to incorporate it into foods as mixtures with other macromolecules. However, there is a dearth of information in the literature regarding the interaction of β-glucan with other commonly utilized commercial gums. Factors such as the processing history, concentration of gum, temperature and pH of the medium, have a profound effect on the stability of the rheological properties of β-glucan in solution

(Bansema, 2000). Moreover, the rheological stability of β-glucan and gum mixtures in aqueous media would strongly depend on the compatibility of the β-glucan and other polysaccharide constituting the system. The limited knowledge of β-glucan-polysaccharide rheology paves the way for further research in this area.

2.9 Human health benefits of β-glucan

In addition to technological properties essential to function as a food ingredient, cereal β-glucans also promote human health. Potential health benefits of cereal β-glucans reported in the literature are lowering of blood cholesterol levels and maintaining cardiovascular health (Jenkins et al., 1985; Anderson and Chen, 1979; Anderson et al., 1992; Keogh et al., 2003), regulation of blood glucose levels for diabetes management (Jenkins et al., 1985; Pins and Kaur, 2006), and regulating anti-cancer activities (Truswell, 1993).

Although the mechanisms by which β-glucans confer hypocholesterolemic activity are not very clearly understood, two probable mechanisms are suggested (Pins and Kaur, 2006): 1) decreased absorption of cholesterol and increased bile acids excretion from gastrointestinal tract; 2) inhibition of cholesterol biosynthesis in liver by the short chain fatty acids produced through colonic fermentation of β glucan. Figure 2-8 presents a schematic of the human gastrointestinal tract showing intestinal digesta containing bile and β-glucan fiber components. Ingestion of β-glucan causes increased viscosity of the intestinal digesta. It is believed that increased viscosity of the digesta can entrap bile acids thus resulting in greater bile acid excretion (Story and Kritchevsky, 1976; Story and Lord, 1987;

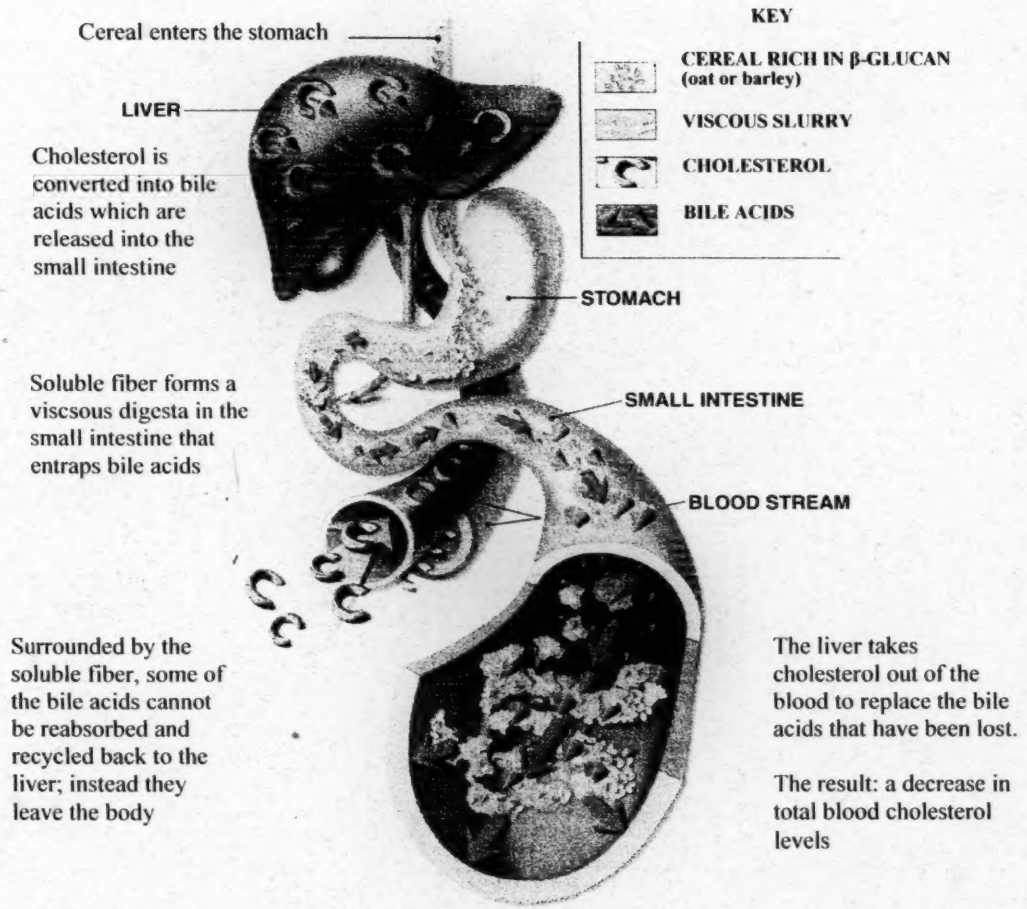

KEY
CEREAL RICH IN β-GLUCAN
(oat or barley)
VISCOUS SLURRY
CHOLESTEROL
BILE ACIDS
Cereal enters the stomach
LIVER
Cholesterol is converted into bile acids which are released into the small intestine
STOMACH
Soluble fiber forms a viscsous digesta in the small intestine that entraps bile acids
SMALL INTESTINE
BLOOD STREAM
Surrounded by the soluble fiber, some of the bile acids cannot be reabsorbed and recycled back to the liver; instead they leave the body
The liver takes cholesterol out of the blood to replace the bile acids that have been lost.
The result: a decrease in total blood cholesterol levels

Figure 2-8. Schematic of a proposed mechanism of action of the cholesterol-lowering properties of β–glucan-rich cereals. Adapted with minor modifications from Marlett et al. (1994).

Bowles et al. 1996). Studies have shown that if fiber viscosity decreases (due to low molecular weight, β-glucanase activity, low solubility, etc.), the potential health benefits get compromised (Frank et al., 2004; Pins and Kaur, 2006). Bile acids secreted into the intestine are normally reabsorbed and returned to the liver where they repress the conversion of cholesterol into bile acids. If bile acids are prevented from returning to the liver by trapping and excretion, increased conversion of cholesterol to bile acids is stimulated, thereby resulting in a reduction in blood cholesterol levels. Furthermore, the lower availability of bile salts in the intestine for micelle formation would also inhibit lipid and cholesterol absorption.

The products of bacterial fermentation of β-glucan have also been reported to play a role in cholesterol metabolism. Bacterial breakdown of dietary fiber in the colon produces short chain fatty acids (SCFA) such as propionate, butyrate etc. These SCFA have been proposed to inhibit the hepatic cholesterol synthesis pathway in the human body (Anderson and Chen, 1979; Anderson et al., 1992; Malkki, 2004).

The mechanism by which β-glucan regulates glucose and insulin metabolism is not fully understood (Pins and Kaur, 2006). However, it is believed that regulation by β-glucan also stems from its ability to form highly viscous digesta thus slowing the absorption of glucose in the intestine and decreasing postprandial blood sugar levels (Marlett, 1997; Yokoyama et al., 1997; Wood et al., 2000). Additional research is required to understand how soluble fibers improve diabetes risk and control.

Consumption of higher levels of fiber has also been positively linked to satiety (Malkki, 2004). Satiety is conferred due to: 1) delayed gastric emptying and slowing nutrient absorption in the small intestine; 2) greater effort and time to ingest food; 3) gastric distention due to excessive production of saliva and acid and increased water absorption; 4) increased transit time within the gut; decreased rate of nutrient absorption and increased gastrointestinal hormone secretions (Howarth et al., 2001; Yao and Roberts, 2001).

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Chapter 3*
STRUCTURAL CHARACTERIZATION OF BARLEY β-GLUCAN MANUFACTURED BY NOVEL FRACTIONATION TECHNOLOGY

3.1 Introduction

β-Glucan is a major soluble dietary fiber in cereal grains such as barley and oats with levels as high as 3 to 8% (Burkus and Temelli, 2000). It has been reported that β-glucan exhibits a wide range of health benefits which includes lowering serum cholesterol, regulating blood glucose levels, and conferring anti-cancer activities (McIntosh et al., 1991; Eastwood, 1992; Newman and Newman, 1992; Morgan et al., 1999). These potential health benefits have led to approval of health claim for oat and barley products in the USA (FDA, 1997 and 2005). In addition to its nutraceutical properties, β-glucan functions as a hydrocolloid, thus contributing to thickening and gelling behavior in food systems. Due to its many physicochemical properties and physiological functions, barley β-glucan has received increasing attention from scientists and product manufacturers.

As depicted in Figure 2-2, barley β-glucan is a linear polysaccharide consisting of *β-(1→4)-* and *β-(1→3)* glycosidic linkages in the ratio of 2.3:1 (Igarashi and Sakurai, 1966; Woodward et al., 1983; Wood et al., 1994). Lichenase, a *(1→3)*, *(1→4)*-β-glucan-4-glucanohydrolase, hydrolyses *(1→4)* linkage of 3-O-subsituted glucose units in β-glucan. Oligosaccharides released by lichenase hydrolysis represent the building blocks of native β-glucan. Analysis of the

* A version of chapter 3 has been submitted to the *Journal of Food Chemistry* for consideration for publication

structural features of intact β-glucan molecule and oligosaccharides released from its lichenase hydrolysis has been previously reported employing various enzymatic and physiochemical techniques (Woodward et al., 1983; Wood and Weisz, 1986; Wood et al., 1989; Wood et al., 1991; Bock et al., 1991). Analysis of oligosaccharides released by lichenase hydrolysis of β-glucan provides detailed structural information.

Extraction methods of producing stable and highly viscous barley β-glucan have been reported in the literature (Wood et al., 1989; Bhatty, 1995; Burkus, 1996; Morgan and Ofman, 1998). As is evident from the previous studies, cost effective production of β-glucan concentrate in terms of yield, β-glucan purity level and viscosity stability of β-glucan concentrate products have been the main concern. Traditional grain fractionation methods for obtaining concentrates utilize aqueous alkali extraction technique. However, this may lead to degradation of β-glucan during processing. A new fractionation technology (Vasanthan and Temelli, 2002) is based on concentrating β-glucan by removal of other grain components and thus β-glucan is recovered in its native form with minimal degradation. However, the impact of this novel technique on the fine structural features of β-glucan is unknown. Therefore, the main objective of the present study was to purify β-glucan from the barley β-glucan concentrate obtained using the novel grain fractionation technique in the laboratory using gentle conditions and to compare its structure with that documented for barley β-glucan in the literature.

3.2 Material and Methods

3.2.1 Materials

A commercial grade of barley β-glucan concentrate (~60% purity) was obtained from Cevena Bioproducts Inc. (Edmonton, AB). Sodium carbonate was from BDH Inc. (Toronto, ON) while HCl was purchased from Fisher Scientific Co. (Nepean, ON). Ethanol was purchased from Commercial Alcohols Inc. (Brampton, ON). Lichenase (EC 3.2.1.73, *Bacillus subtilis*) was obtained from Megazyme International Ireland ltd. (Wicklow, Ireland). Authentic glucose, maltose, maltotriose (DP3), maltotetraose (DP4), maltopentaose (DP5), maltohexaose (DP6), maltoheptaose (DP7) and 2,5-dihydroxybenzoic acid (DHB) and 3-hydroxy 4-methoxy benzoic acid (HMB) were procured from Sigma Chemical Company (St. Louis, MO).

3.2.2 Extraction and purification of β-glucan

Barley β-glucan concentrate (~60% β-glucan purity) was used as the starting material for the further purification performed at laboratory scale. The laboratory scale purification procedures were based on non-alkali and non-enzymatic extraction. The method involved the solublization of β-glucan in deionized Milli-Q water at 82°C followed by centrifugation, collecting the supernatant and separating β-glucan in supernatant by alcohol precipitation and drying overnight at 40°C.

3.2.3 Chemical analyses

Contents of moisture, β-glucan, starch, and protein (N x 6.25) of dried samples were determined in duplicate according to the methods of AACC (1983), McCleary and Glennie-Holmes (1985), Holm et al. (1986) and FP-428 Nitrogen Determinator (Leco Corp., St. Joseph, MI), respectively.

3.2.4 Lichenase hydrolysis of laboratory purified β-glucan

A solution (0.5% w/w) of dried purified β-glucan was prepared by stirring continuously while incubating in a boiling water bath for 1 h or until β-glucan was fully solubilized. After cooling to room temperature, the pH was adjusted to 6.5 followed by the addition of 5 mL of liquid lichenase (150 U). The mixture was incubated at 50°C for 1 h with vortexing at every 20 min. The resulting hydrolysate was heated in a boiling water bath to inactivate the enzyme and centrifuged at 6000xg for 15 min. The supernatant was collected and subjected to analysis by instrumental techniques.

3.2.5 HPLC chromatography of hydrolysate

An HPLC system equipped with a Varian 9010 Solvent delivery system, an HP series 1050 auto sampler, a Jordi Gel DV 13 polyamine column (250 mm length x 4.6 mm i.d.; Bellingham, MA), and an evaporative light scattering detector (Alltech 500 ELSD, Mandel Scientific Company Ltd., Guelph, ON) was used. Two eluents, namely HPLC grade water (A) and acetonitrile (B), were used as mobile phase. The solvent gradient used was 10% (v/v) A and 90% (v/v) B for the first 18

min, 22% (v/v) A and 78% (v/v) B from 18-36 min, and 90% (v/v) A and 10% (v/v) B after 36 min. The detector temperature was set at 125°C and a flow rate of 1.0 mL/min was maintained. Hundred microliters from each of the standard DP3 to DP7 oligosaccharide solutions were mixed in a vial and the HPLC retention times were recorded. Retention times for individual oligosaccharide standards were used for the identification of oligosaccharides in the mixture. During the HPLC purification of oligosaccharides present in the β-glucan hydrolysate, injection volume was maintained at 50 µL to ensure a better resolution of the compounds with a good baseline.

3.2.6 MALDI TOF-MS of hydrolysate and HPLC fractions

Matrix assisted laser desorption ionization-time of flight-mass spectrometry (MALDI-TOF-MS) was performed using the Bruker Proflex III linear mode unit (Bruker Analytical Systems Inc., Billerica, MA). A combination of 2,5-dihydroxybenzoic acid (DHB) and 3-hydroxy-4-methoxy benzoic acid (HMB) was used as a matrix in the ratio of 9:1 for crystallization of β-glucan hydrolysates and oligosaccharides collected by HPLC fraction collector. The nitrogen laser pulse was accelerated under 20 kV with delayed extraction before entering the time-of-flight mass spectrometer (Jiang and Vasanthan, 2000).

3.2.7 ^{1}H and ^{13}C NMR spectroscopy

High-resolution ^{1}H and ^{13}C NMR spectra were recorded in D_2O at 498 MHz and 125.3 MHz, respectively, on a Varian Unity 500 MHz NMR spectrometer (Palo

Alto, CA) operating at 27°C. Homonuclear $^{1}H/^{1}H$ correlation spectroscopy (COSY, GCOSY, TOCSY, TROESY) for DP3 and DP4 fractions were performed at 599.93 MHz at 27°C. Heteronuclear $^{13}C/^{1}H$ correlation experiments (HETCOR, HMBC) were run at 499.83 MHz at 27°C. ^{1}H resonances were measured relative to internal acetone (2.22 ppm, DOH at 4.75 ppm at 27°C). ^{13}C resonances were referenced to 1% acetone at 31.07 ppm at 27°C.

3.2.8 Statistical analysis

Purification and hydrolysis experiments were performed in duplicates. All the chemical analyses were performed in duplicates for the purified concentrate fiber sample. NMR, MALDI-TOF, and HPLC analyses were performed in duplicate for each of the purified and hydrolyzed-purified samples.

3.3 Results and Discussion

3.3.1 Proximate composition of purified and crude barley β-glucan concentrate

The proximate composition of barley β-glucan concentrate and laboratory purified barley β-glucan is shown in Table 3-1. The recovery, defined as the ratio between the amount of β-glucan in purified sample and the amount of β-glucan present in the β-glucan concentrate, for the laboratory purification method was 78.3% (± 1.3) (w/w). Lipid content was 0% (w/w) in the barley β-glucan concentrate used and hence it was assumed that the purified barley β-glucan contains no lipids.

Table 3-1 Proximate composition of barley β-glucan concentrate and laboratory purified barley β-glucan

Parameter (%, w/w)	Barley β-glucan Concentrate	Barley β-glucan (after purification)
β-Glucan	61.70 ± 1.50	82.50± 0.90
Moisture	8.20 ± 0.29	5.40± 0.21
Protein	6.60 ± 0.11	2.70 ± 0.08
Lipids	0.0	0.0
Starch	8.80± 0.25	3.20 ± 0.11
Ash	4.50 ± 0.20	2.10 ± 0.01
Others*	7.89 -11.20	2.79 - 4.10

* insoluble dietary fiber, vitamins etc.

3.3.2 ^{13}C NMR spectroscopy of purified native β-glucan

Figure 3-1 shows ^{13}C NMR spectrum of purified barley β-glucan isolated from barley β-glucan concentrate. The ^{13}C NMR assignments are shown in Table 3-2 and compares well with those reported previously in literature (Bock et al., 1991; Bernabe et al., 1994; Cui et al., 2000). The resonance at 102.8 ppm was assigned to C-1 carbon of a (1→3)-β-linkage. The resonance at 102.6 ppm was due to C-1 carbon of a (1→4)-β-linkage. The C-3 carbon of a (1→3)-β-linkage appeared at 84.1 ppm. C-3 resonance in O-3 substituted residue occurs singly, suggesting that most of the (1→3) linkages occur singly (Wood, 1993). The resonance at 73.6, 68.3, 75.8 and 61.0 ppm were assigned to the remaining carbons, C-2, C-4, C-5 and C-6 of the O-3-β-glucopyranosyl residue, respectively, while those at 73.3, 74.4, 78.7, 75.1, and 60.4 ppm were assigned to C-2, C-3, C-4, C-5 and C-6 carbons of the O-4-β-glucopyranosyl residue, respectively. C-1, C-3, C-4, C-5 and C-6 carbons of the O-4-β-glucopyranose-3-O residue resonated at 102.8, 74.4, 78.7, 75.1 and 60.4 ppm, respectively. C-2 resonance of O-4-β-glucopyranose-3-O residue was not well resolved in the spectrum and was not reported. The minor discrepancy in resonance frequencies may be attributed to the use of β-glucanase and cellulase in addition to lichenase by other researchers (Bock et al., 1991). The ratio between β-(1→3) to β-(1→4) linkages of native barley β-glucan was 1:2.3 which was similar to those reported in earlier NMR investigations (Bock et al., 1991, Wood, 1993). ^{13}C NMR spectra for barley β-glucan purified from barley β-glucan concentrate was identical to that reported in the literature (Fig. 3-1).

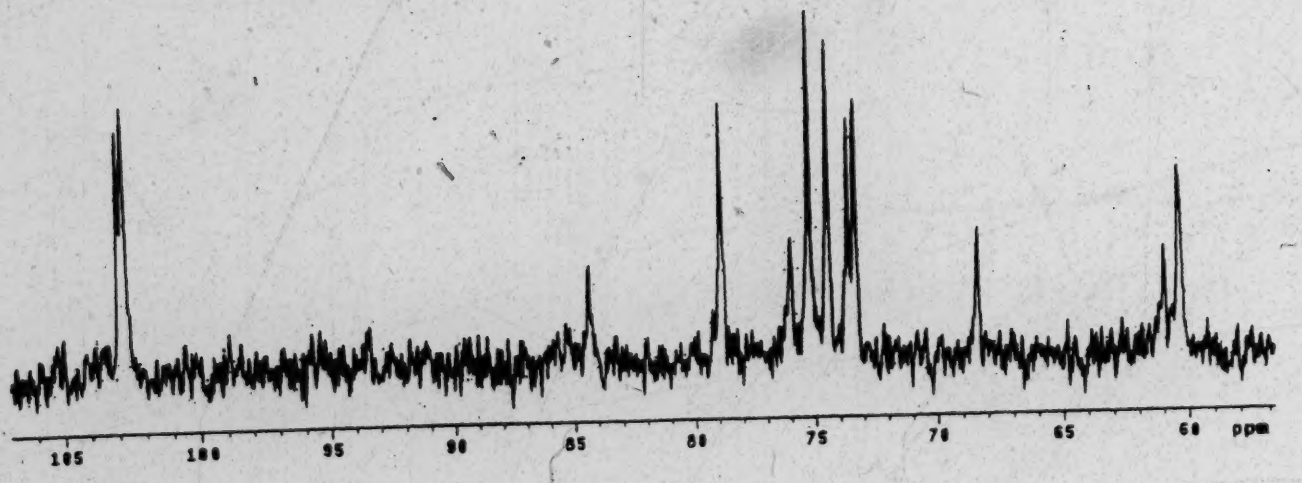


Figure 3-1 ^{13}C NMR spectra of purified barley β-glucan

Table 3-2 ^{13}C NMR assignments for purified barley β-glucan

	^{13}C chemical shifts, ppm					
Glucosyl residue (Glc *p*)	**C-1**	**C-2**	**C-3**	**C-4**	**C-5**	**C-6**
O-3-β-Glc *p*	102.6	73.6	84.1	68.3	75.8	61.0
O-4-β-Glc *p*	102.6	73.3	74.4	78.7	75.1	60.4
O-4-β-Glc *p*-3-O	102.8	x	74.4	78.7	75.1	60.4

3.3.3 Lichenase hydrolysis of purified β-glucan and HPLC separation of fragments

Figure 3-2 depicts the HPLC chromatogram of hydrolyzed β-glucan. Two major fragments resolved at retention times of 15.51 and 19.37 and they were tentatively identified as *DP*3 and *DP*4, respectively. The *DP*5 and several minor fragments (*DP*5-11) were pooled together since the resolution of these components on the HPLC column used was not satisfactory. The ratio between *DP*3 and *DP*4 fragments was 2.3:1 which is in agreement with the ratio reported by Jiang and Vasanthan (2000) for β-glucan from regular barley varieties (e.g., Candle).

3.3.4 MALDI TOF- mass spectroscopies of β-glucan hydrolysate

The MALDI-TOF spectrum of β-glucan hydrolysate (Figure 3-3) confirmed the presence of *DP*3 to *DP*11 species with *DP*3 and *DP*4 being the predominant species. This indicates that *DP*3 and *DP*4 units are the main building blocks of barley β-glucan. DP5 to *DP*11, which were not detected in the HPLC chromatogram, were detected in MALDI-TOF MS (Fig. 3-3). This result is in agreement with the findings in earlier barley β-glucan structural studies (Woodward et al., 1983; Wood et al., 1991; Wood et al., 1994; Jiang and Vasanthan, 2000).

3.3.5 MALDI-TOF and ^{1}H and ^{13}C NMR spectroscopies of isolated fragments

MALDI-TOF MS of tentatively identified DP3 HPLC fraction showed a dominant ion at m/z of 526.88, which correspond to a sodium salt of a β-glucan fragment with three glucopyranosyl residues (Fig. 3-4). Four more unidentified sodium salts of minor oligosaccharides appeared at m/z of 637.02, 646.63, 702.72

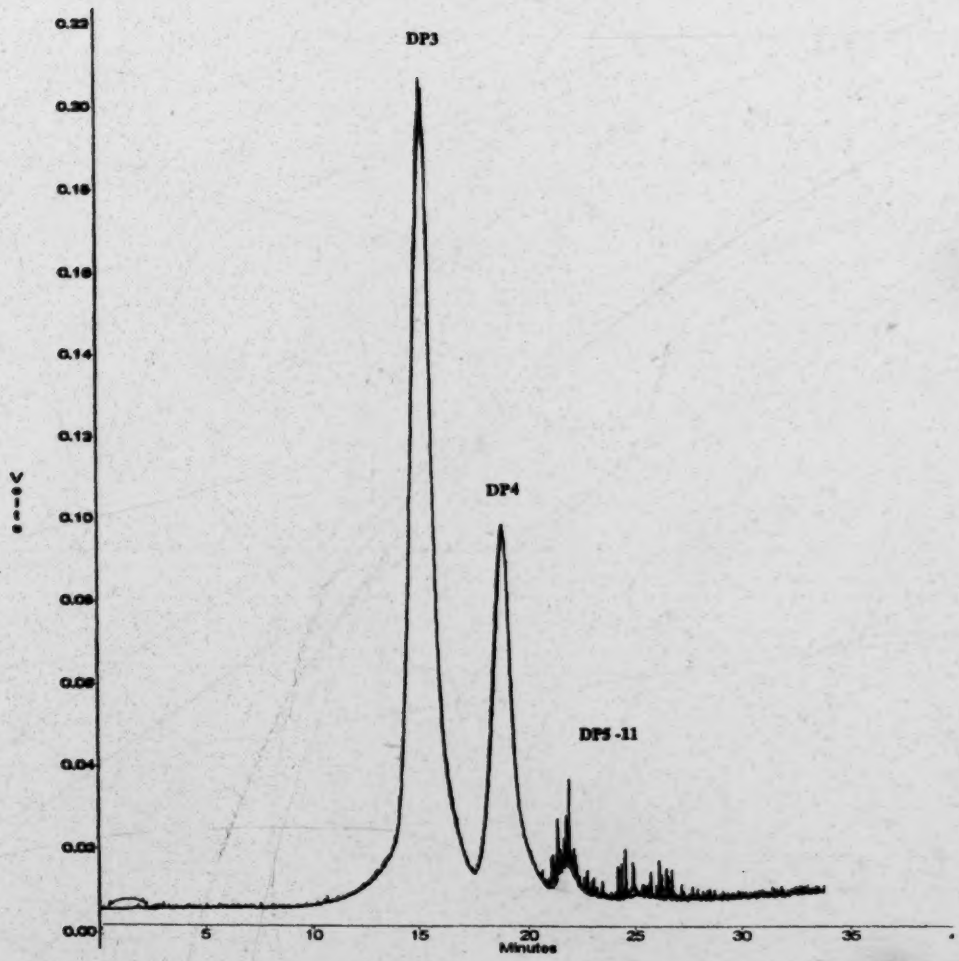
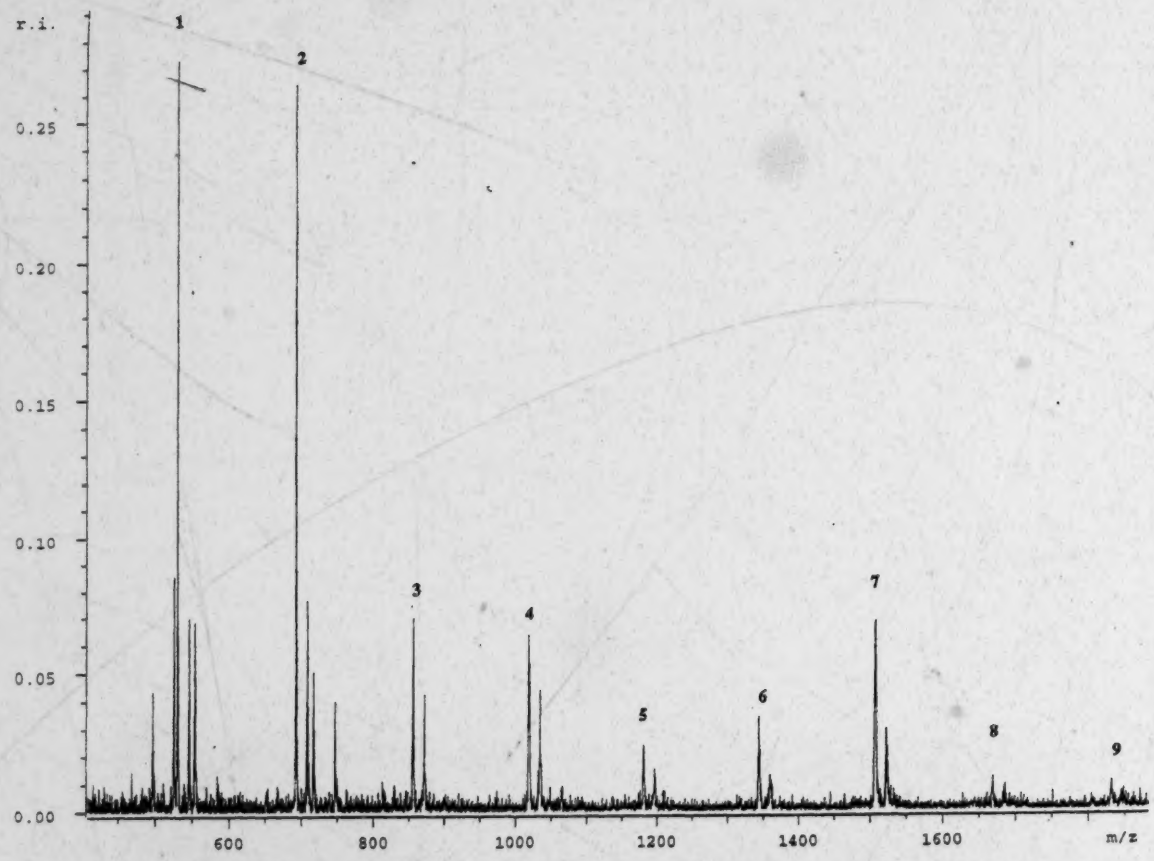


Figure 3-2 HPLC chromatogram of barley β-glucan hydrolysate.



1. [*DP* 3 + Na]+ ; 2. [*DP* 4 + Na]+ ;3. [*DP* 5 + Na]+ ; 4. [*DP* 6 + Na]+ ; 5. [*DP* 7 + Na]+ ;
6. [*DP* 8 + Na]+ ;7. [*DP* 9 + Na]+ ; 8. [*DP* 10 + Na]+ ; 9. [*DP* 11 + Na]+.

Figure 3-3 MALDI-TOF mass spectrum of barley β-glucan hydrolysate

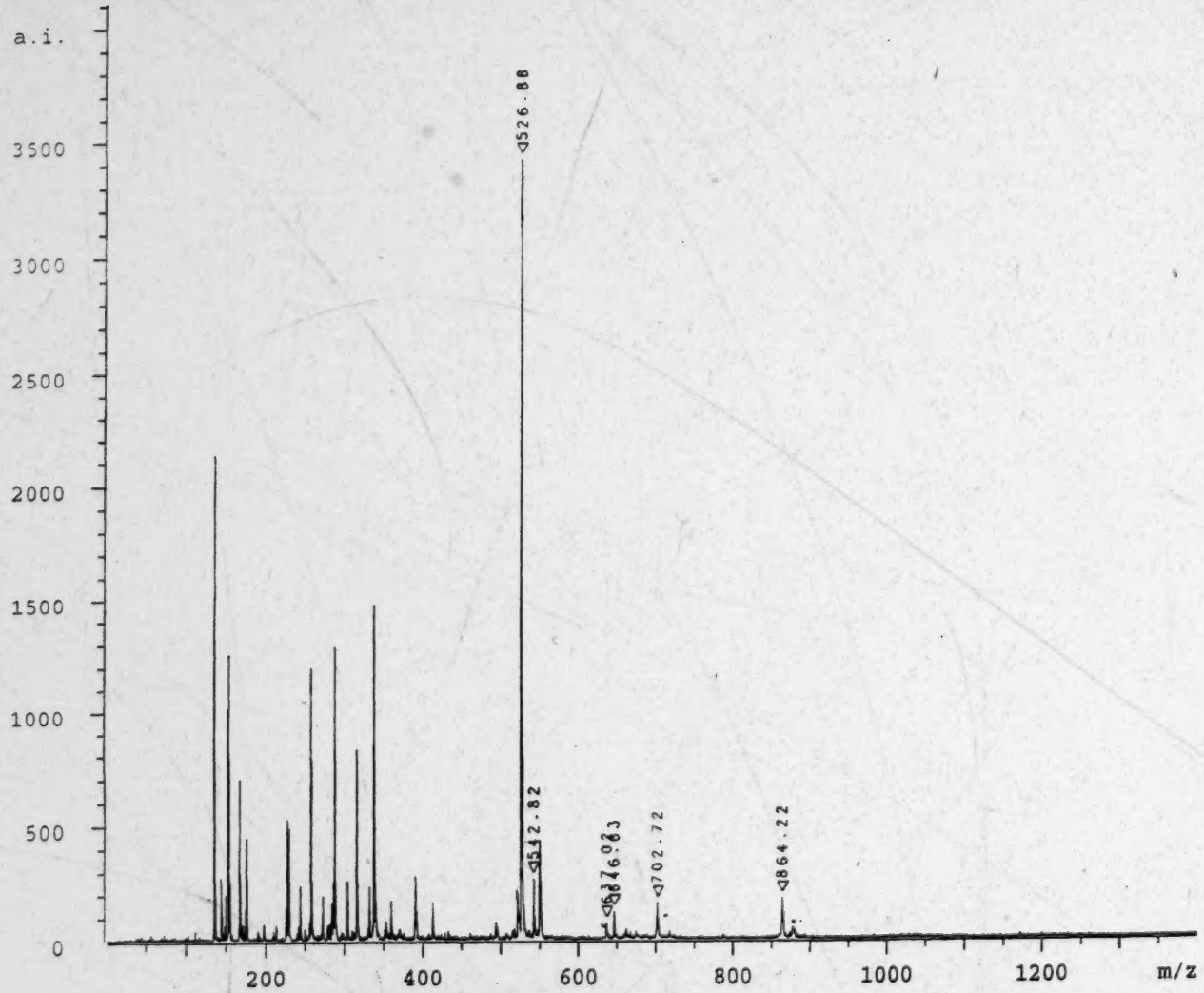


Figure 3-4 MALDI-TOF mass spectrum of *DP*3 isolated using HPLC fraction collection from β-glucan hydrolysate.

and 864.22. The major ion appeared at m/z of 688.35 of MALDI-TOF mass spectrum of tentatively identified *DP*4 HPLC fraction corresponded to a sodium salt of a β-glucan fragment with four glucopyranosyl residues while two unidentified fragments appeared at m/z of 863.91 and 1052.31 (Fig. 3-5). Potassium salt counterparts of all β-glucan fragments were also present. The specificity of lichenase enzyme favors the cleavage of specific glycosidic bonds (as shown in Fig. 3-6) of β-glucan making *DP*3 and *DP*4 fragments the dominant species in the hydrolysate. Therefore, the structure of β-glucan concentrated using the novel technology consisted of two predominant oligosaccharides, *DP*3 and *DP*4 (Jiang and Vasanthan, 2000). The monosaccharide units of *DP*3 are designated as A-B-C (Fig. 3-4), whereas the monosaccharide units of *DP*4 are designated as A-B-C-D starting from non-reducing end. ^{13}C NMR spectrum showed DP3 to be a trisaccharide as it showed 24 signals. The 24 signals were due to the presence of 6-carbon in each of 3-glucopyranosyl rings constituting a trisaccharide and a proportion of α-form formed due to mutarotation. This is in agreement with the observations of Bock et al. (1991). ^{13}C chemical shifts of *DP*3 and *DP*4 isolated using HPLC are shown in Table 3-3 whereas Table 3-4 shows ^{1}H chemical shifts for the isolated residues. The ^{1}H spectrum showed three well resolved doublets for the anomeric protons of β-glucan residues, which are similar to those reported by Cui et al. (2000). Heteronuclear correlation ($^{13}C/^{1}H$) NMR allowed the assignment of protons to respective carbons of DP3 and DP4. ^{1}H and ^{13}C assignments of

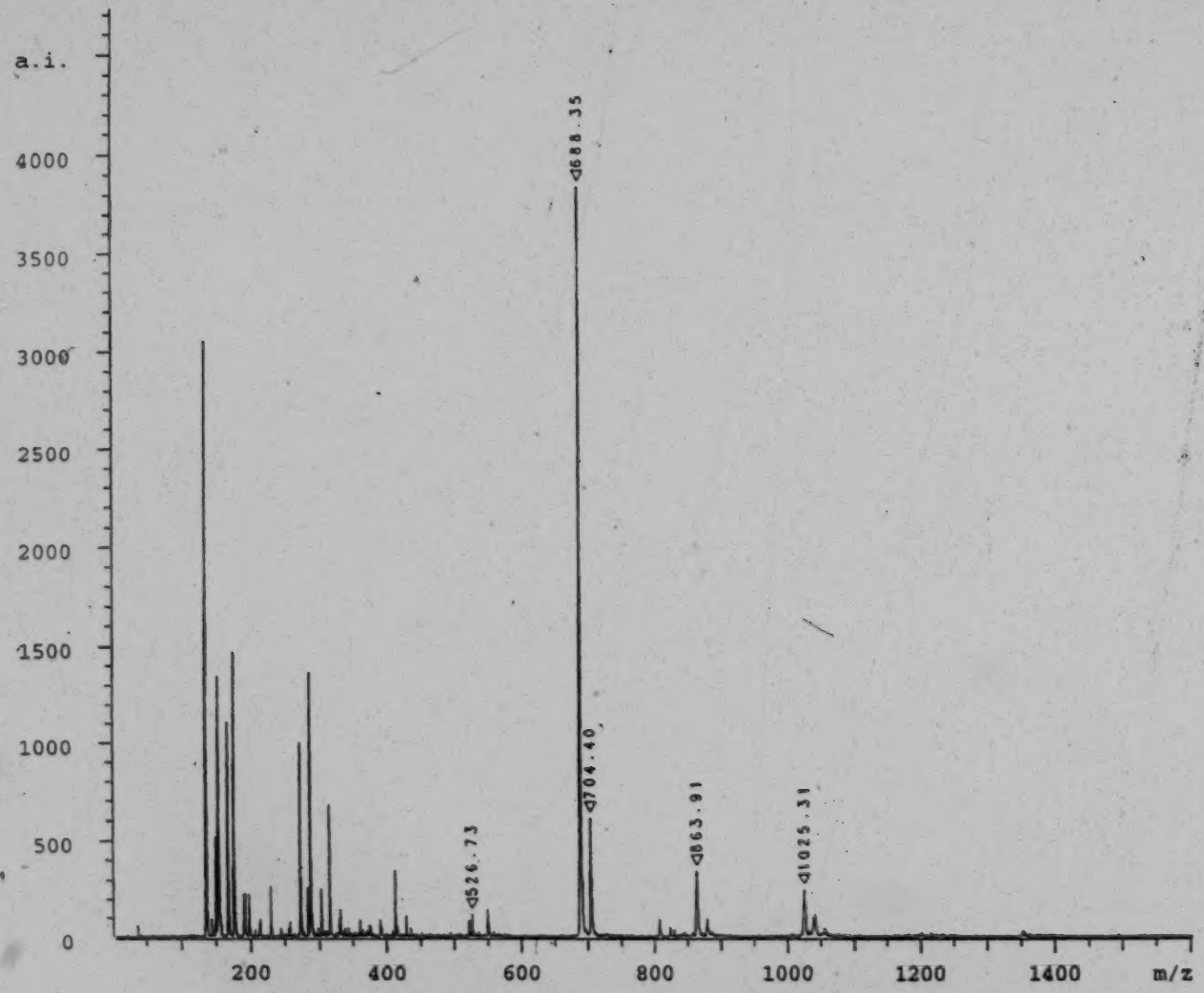


Figure 3-5 MALDI-TOF mass spectrum of *DP*4 isolated using HPLC fraction collection from β-glucan hydrolysate.

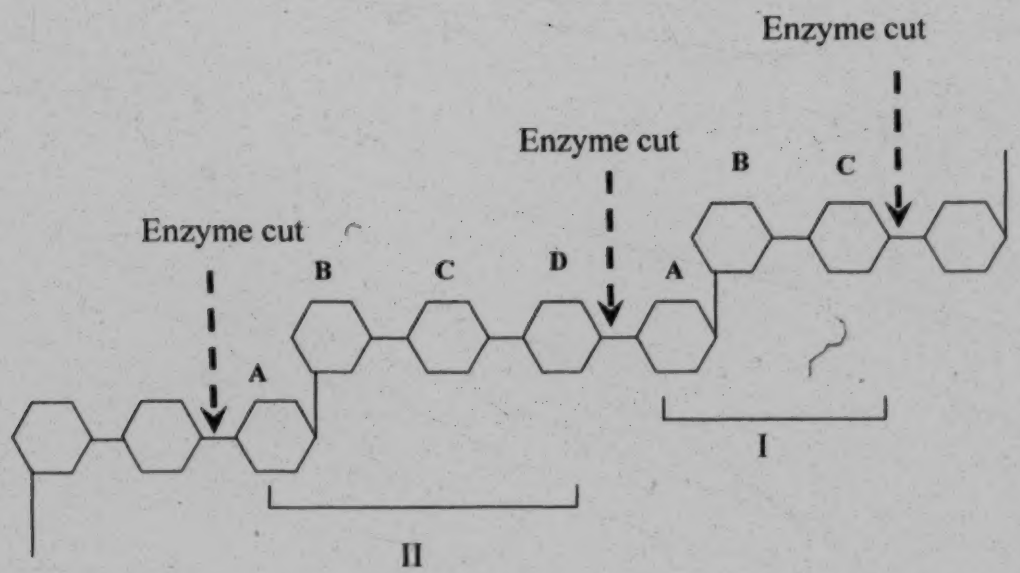


Figure 3-6 Schematic showing the mechanism of lichenase action on *(1→3)*, *(1→4)*-β-glucan.

Note: Arrows show the points where lichenase cleaves the linkages.

Table 3-3 ^{13}C assignments of oligosaccharides derived from barley β-glucan

Glucosyl residue (Glc *p*)		^{13}C chemical shifts, ppm					
		C-1	C-2	C-3	C-4	C-5	C-6
Trisaccharide							
O-3-β-Glc *p*	Residue A (α)	92.8	71.8	83.2	68.9	72.1	60.8
	Residue A (β)	96.5	74.5	85.3	69.0	76.4	60.8
O-4-β-Glc *p*	Residue B	103.4	74.2	75	79.4	75.6	61.6
β-Glc *p*	Residue C	103.3	74.1	76.4	70.1	76.7	61.4
Tetrasaccharide							
O-3-β-Glc *p*	Residue A (α)	92.7	71.7	83.0	68.8	71.9	61.4
	Residue A (β)	96.4	74.8	85.3	85.3	68.8	61.4
O-4-β-Glc *p*	Residue B	103.4	74.3	75.5	79.2	76.2	60.7
O-4-β-Glc *p*	Residue C	103.1	73.6	75.5	79.2	76.2	60.7
β-Glc *p*	Residue D	103.3	74.0	76.3	70.2	76.7	61.4

Table 3-4 ^{1}H assignments of oligosaccharides derived from barley β-glucan

Glucosyl residue (Glc *p*)		^{1}H chemical shifts, ppm H-1	H-2	H-3	H-4	H-5	H-6 (A)	H-6 (B)
Trisaccharide								
O-3-β-Glc *p*	Residue A (α)	5.23	3.72	3.91	3.53	3.89	3.91	3.77
	Residue A (β)	4.65	3.43	3.75	3.52	3.52	3.92	3.73
O-4-β-Glc *p*	Residue B	4.75	3.40	3.67	3.66	3.63	x	x
β-Glc *p*	Residue C	4.49	3.31	3.52	3.40	3.52	3.91	3.73
Tetrasaccharide								
O-3-β-Glc *p*	Residue A (α)	5.22	3.74	3.91	3.55	3.89	3.89	3.77
	Residue A (β)	4.65	3.46	3.76	3.55	3.55	3.91	3.75
O-4-β-Glc *p*	Residue B	4.75	3.41	3.69	3.69	3.64	4.01	3.84
O-4-β-Glc *p*	Residue C	4.52	3.41	3.70	3.71	3.66	x	x
β-Glc *p*	Residue D	4.51	3.32	3.49	3.42	3.49	3.91	3.75

trisaccharide and tetrasaccharide are in complete agreement with the original assignments reported by Bernabe et al. (1994) and Bock et al. (1991).

3.4 Conclusions

The fine structure of barley β-glucan purified in the laboratory from barley β-glucan concentrate is similar to those of barley β-glucan reported in the literature. NMR spectroscopy of oligosaccharides released by lichenase hydrolysis of barley β-glucan provided detailed structural information. As reported in earlier NMR and HPLC investigations, the ratio of β-(1→3) to β-(1→4) linkages in the intact β-glucan molecule purified from barley β-glucan concentrate is 2.3:1.

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Chapter 4*
^{31}P-NUCLEAR MAGNETIC RESONANCE (NMR) SPECTROSCOPIC ANALYSIS OF PHOSPHORUS IN OAT AND BARLEY β-GLUCANS

4.1 Introduction

Oat and barley β-glucans are important biopolymers that confer physicochemically and nutraceutically important properties (McIntosh et al., 1991; Morgan et al., 1999; Eastwood, 1992; Newman and Newman, 1992). These biopolymers are integral components of cell walls of *Poaceae* family and are particularly associated with the cell walls of oat and barley cereal grains (Morgan et al., 1999). Many of the important properties of β-glucans are directly linked to their molecular features. Mixed-linkage β-glucans mainly consist of cellotriosyl and cellotetraosyl residues separated by single (1→3)-β-linkages (Igarashi and Sakurai, 1966; Wood et al., 1991; Cui et al., 2000). Enzymatic and spectroscopic techniques have shown that the cellotriosyl and cellotetraosyl residues of oat and barley β-glucans are arranged randomly in the ratio of 2.2:1 and 3.3:1, respectively (Cui et al., 2000). These ratios are important in terms of solubility and rheological properties of β-glucans.

To date, β-glucans have been described as neutral biopolymers in the literature. The solubility characteristics of oat and barley β-glucans, however, may suggest otherwise. For example, the gelling properties of various starches, close counterparts of β-glucans, are linked to their association with various forms of

* A version of chapter 4 has been submitted to *Food Hydrocolloids* for consideration for publication

phosphorus that makes the polymer molecules negatively charged. For instance, Lim et al. (1994) reported the association of phospholipids, phosphoproteins and esterified phosphates (phosphate monoesters), which affects the water-binding capacity and clarity of starch paste. It has been shown that lysophospholipids form helical complexes with starch, which in turn reduces the water-binding capacity of starch paste while increasing paste opaqueness (Schoch, 1942; Swinkles, 1985; Morrison et al., 1990). Phosphate monoesters, however, promote the hydrophilic nature of starch paste by increasing the repulsion between starch chains with negatively charged phosphate groups, thus enhancing water-binding capacity, swelling, and paste clarity (Lim, 1990).

In this study, it was hypothesized that phosphorus is associated with β-glucan in the form of phospholipids, phosphoproteins, and phytates and/or phosphate monoesters. To test this hypothesis, the objectives were: (1) to isolate and purify β-glucan from oat and barley β-glucan concentrates, (2) to quantify phosphorus content of the crude and purified β-glucan concentrates, and (3) to determine the nature of phosphorus in β-glucans by phosphorus nuclear magnetic resonance (^{31}P-NMR) spectroscopy.

4.2 Materials and Methods

4.2.1 Materials

Oat and barley β-glucan concentrates, a concentrated form of β-glucan (45-65%, w/w, β-glucan) were provided by Cevena Bioproducts Inc. (Edmonton, AB).

Concentrated hydrochloric (HCl) and nitric acids (HNO_3) were purchased from Fisher Scientific Co. (Nepean, ON). Ethanol was purchased from Commercial Alcohols Inc. (Brampton, ON) while Termamyl 120 LN, a thermostable α-amylase (E.C. 3.2.1.1) of *Bacillus licheniformis*, was from Novo Nordisk BioChem, Inc. (Toronto, ON). Lichenase (EC 3.2.1.73, *Bacillus subtilis*) was obtained from Megazyme International Ireland Ltd. (Wicklow, Ireland). Ammonium molybdate [$(NH_4)_6Mo_7O_{24}.4H_2O$], ammonium metavandate (NH_4VO_3), sodium carbonate and zinc acetate were purchased from BDH Inc. (Toronto, ON).

4.2.2 Extraction and purification of β-glucan from oat and barley β-glucan concentrates

The purification of β-glucan from oat and barley β-glucan concentrates was based on the methods of Burkus (1996) and Lekhi (2003) with modifications. Figure 4-1 depicts the laboratory scale method used for β-glucan isolation from β-glucan concentrate. The method involved the solubilization of β-glucan in boiling deionized Milli-Q water, treatment with thermostable α-amylase (added at a rate of 1%, w/w, of available starch in the sample), followed by protein denaturation and subsequent alcohol-assisted precipitation of β-glucan. Purified β-glucan concentrate obtained was then dried overnight at 50°C.

4.2.3 Lichenase hydrolysis of β-glucan

A solution of purified β-glucan (0.5 %, w/w) was prepared in deionized Milli-Q water. Solution was stirred continuously while incubating in a boiling water

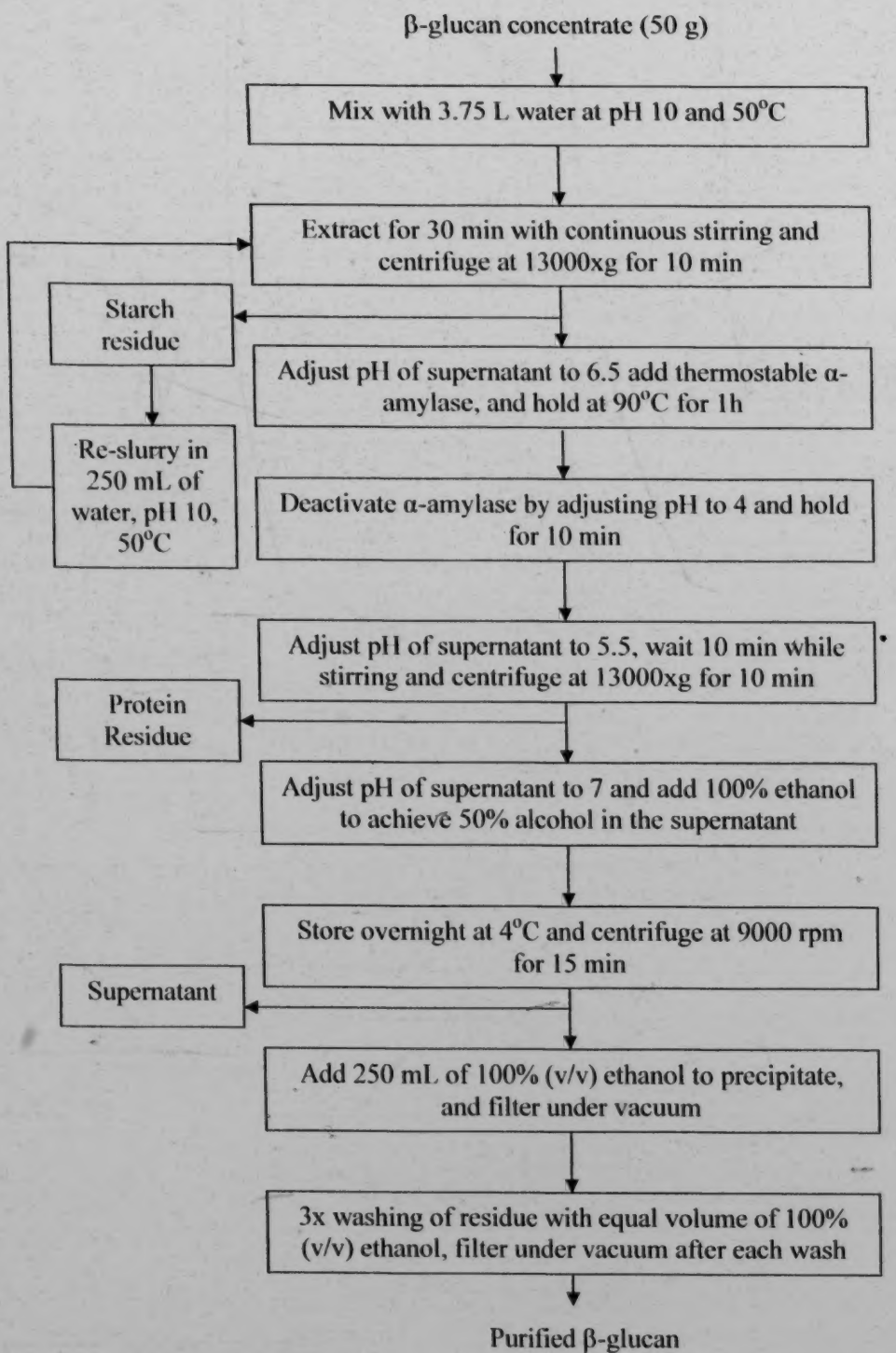
β-glucan concentrate (50 g)
Mix with 3.75 L water at pH 10 and 50°C
Extract for 30 min with continuous stirring and centrifuge at 13000xg for 10 min
Starch residue
Re-slurry in 250 mL of water, pH 10, 50°C
Adjust pH of supernatant to 6.5 add thermostable α-amylase, and hold at 90°C for 1h
Deactivate α-amylase by adjusting pH to 4 and hold for 10 min
Adjust pH of supernatant to 5.5, wait 10 min while stirring and centrifuge at 13000xg for 10 min
Protein Residue
Adjust pH of supernatant to 7 and add 100% ethanol to achieve 50% alcohol in the supernatant
Store overnight at 4°C and centrifuge at 9000 rpm for 15 min
Supernatant
Add 250 mL of 100% (v/v) ethanol to precipitate, and filter under vacuum
3x washing of residue with equal volume of 100% (v/v) ethanol, filter under vacuum after each wash
Purified β-glucan

Figure 4-1 Flow-chart depicting the steps involved in the β-glucan purification method at laboratory scale

bath for 1 h or until β-glucan was fully solubilized. After cooling to ambient temperature, the pH was adjusted to 6.5 followed by the addition of 0.2 mL of liquid lichenase consisting of 200 U. The mixture was incubated at 50°C for 1 h with vortexing at every 20 min. The resulting hydrolysate was heated in a boiling water bath to inactivate the enzyme and centrifuged at 13000xg for 15 min. The supernatant was collected, lyophilized, re-dissolved in deuterated water (D_2O) and subjected to ^{31}P NMR spectroscopy.

4.2.4. Chemical analyses

Content of moisture, β-glucan, starch, and protein of dried samples was determined in duplicate according to the methods of AACC (1983), McClearly and Glennie-Holmes (1985), Holm, Bjorck, Drews, and ASP, 1986) and FP-428 Nitrogen Determinator (Leco Corp., St. Joseph, MI), respectively.

4.2.5. Determination of total phosphorus content

Phosphorus analysis of oat/barley β-glucan concentrates and purified oat/barley β-glucan was performed on duplicate samples using the molybdate blue method of Smith and Caruso (1964). Standard curve for phosphate determination was established using standard KH_2PO_4 solutions having concentrations of 0.001-12 mg/25 mL. Ten milliliters of 10% (w/v) zinc acetate were added over the sample (3 g) in 50 mL crucible and evaporated to dryness on a steam bath. Sample was then charred on a hot plate, the residue ignited in a muffle furnace for 2h at 550°C, cooled to room temperature and 3 mL of 29% (v/v) HNO_3 were added. Contents were dried

on a steam bath, charred, ignited for 1 h at 550°C, cooled to room temperature and the dish sides were washed with 5 mL of 29% (v/v) HNO_3. Water (7.5 mL) was added to the crucible that was then covered with a watch glass, heated to boiling, held for 10 min and cooled to room temperature. Contents were then quantitatively filtered through Whatman # 1 filter paper into a 50 mL volumetric flask, residue washed with four 5 mL aliquots of water and filled upto the mark with water. An aliquot containing < 2.5 mg of phosphorous was pipetted into a 100 mL volumetric flask, 10 mL of 29% (v/v) HNO_3 added and mixed. Ten milliliters of 5 % (w/v) ammonium molybdate were added, mixed, filled up to the mark with water and mixed again. Absorbance at 460 nm was measured after allowing the solution to stand for 10 min. The degree of substitution (DS) was calculated using the equation of Smith and Caruso (1964) that is:

$$DS = \frac{162P}{3100 - 102P}$$

where P = % phosphorous (w/w, dry basis) of the phosphorylated β-glucan

4.2.6 Determination of phytate phosphorous content

Phytate phosphorus analysis of oat/barley β-glucan concentrates and purified oat/barley β-glucan was performed on duplicate samples using AOAC official methods (AOAC 2000).

4.2.7 ^{31}P NMR of hydrolysate

The lyophilized hydrolysate (350 mg) was re-dissolved in 0.7 ml of D_2O by heating at 90°C until a homogeneous solution was obtained. ^{31}P-NMR analyses on homogeneous solutions were performed on a Varian Inova 400 MHz spectrometer and spectra were acquired at a frequency of 161.935 MHz with 30,000 scans per sample. The chemical shifts were recorded with ^{1}H decoupled settings at 27°C. All chemical shifts were reported in ppm and referenced with respect to H_3PO_4.

4.2.8 Determination of solubility characteristics of oat and barley β-glucans

Solubility of oat/barley β-glucan concentrates and purified oat/barley β-glucan was performed in duplicate samples using an *in vitro* physiological extraction procedure at 37°C that simulated human gut environment (Beer et al., 1997).

4.2.9 Statistical analysis

Both oat and barley β-glucan samples were purified in duplicate from respective concentrates. Chemical analysis for each of the oat and barley β-glucan samples purified in the laboratory were performed in duplicates. Purified β-glucan hydrolysates were prepared from each of the purified oat and barley β-glucan and two samples from each hydrolysates prepared were evaluated by ^{31}P-NMR spectroscopy.

4.3 Results and discussion

4.3.1 Recovery, purity and chemical composition of isolated β-glucans

The recovery is defined as the ratio between the amount of β-glucan in purified sample and the amount of β-glucan present in the β-glucan concentrate. The recovery obtained for oat and barley β-glucan was 83.1 and 84.3% (w/w) (Table 4-1), respectively. β-Glucan content of oat and barley concentrate was 40.6% and 65.2% (w/w, dry basis), respectively. Regardless of its β-glucan content, oat β-glucan concentrate yielded high purity β-glucan (97%, w/w) as compared to the purity of 90% (w/w) β-glucan obtained from barley β-glucan concentrate after the purification treatment (Table 4-1).

Contents of moisture, starch, protein and lipid in the purified β-glucans obtained from oat and barley β-glucan concentrate is shown in Table 4-1. Lipid content was 0% (w/w) in the barley starting material used and hence it was assumed that the purified barley β-glucan contains no lipids whereas the purified oat β-glucan contained 0.5% (w/w) lipids (Table 4-1).

4.3.2 Phosphorus content

Table 4-2 shows the β-glucan, and total and phytate phosphorus contents of oat and barley β-glucan concentrates and the corresponding laboratory purified β-glucans. The phytate and total phosphorus contents of oat β-glucan concentrate were 0.745% and 0.92% (w/w), respectively. However, the phytate and total phosphorus

Table 4-1 Recovery and composition of purified β-glucan obtained from barley and oat β-glucan concentrates

(% w/w, dry basis)	Oat β-glucan	Barley β-glucan
β-glucan	97±0.8	90±1.1
Recovery	83.1±1.0	84.3±0.85
Moisture	4±0.2	4.4±0.14
Proteins	1.4±0.04	1.7±0.02
Lipids	0.5±0.03	0
Starch	1.1±0.02	0.9±0.01

Table 4-2 Total and phytate phosphorous contents of β-glucan concentrates and purified β-glucans.

Sample	β-Glucan (%, w/w)	Phosphorous (%, w/w)		
		Total	Phytate	Others[3]
Oat β-glucan concentrate	40.6±0.88	0.920±0.040	0.745±0.030	0.175
Barley β-glucan concentrate	65.2±1.20	0.170±0.006	0.103±0.005	0.067
Purified oat β-glucan[1]	97.0±1.00	0.201±0.010	0.0	0.201
Purified barley β-glucan[2]	90.0±1.10	0.092±0.007	0.0	0.092

[1] purified from oat β-glucan concentrate
[2] purified from barley β-glucan concentrate
[3] Other Phosphorous = Total Phosphorous – Phytate Phosphorous

contents of barley β-glucan concentrate were 0.103% and 0.170 % (w/w), respectively. Therefore, the phytate and total phosphorus contents of oat β-glucan concentrate were 7.2 and 5.4-fold, respectively, higher than those in barley β-glucan concentrate. For purified oat β-glucan, the total phosphorous content was 0.201% (w/w, DS=0.011) in contrast to the total phosphorus content of 0.920% (w/w) in oat β-glucan concentrate. Mathematically, this implies that 21.8% of total phosphorous in oat β-glucan concentrate was associated with β-glucan while the rest was bound to the other components present. Similarly, the association of phosphorus with barley β-glucan amounted to 54.1% of total phosphorus in barley β-glucan concentrate and the remainder was associated with other components. Although both types of β-glucan concentrates contained considerable amount of phytate phosphorous, the purified β-glucan did not contain this form of phosphorus in detectable quantities (Table 4-2).

4.3.3 ^{31}P NMR evidence for the presence of phosphate monoesters

One dimensional ^{31}P NMR spectroscopy has been successfully employed to determine the nature of phosphorus associated with grain and tuber starches (Takeda and Hizukuri, 1982; Muhrbeck et al., 1991; Muhrbeck and Nantes, 1991; Lim and Seib, 1993; Wood, 1993; Lim et al., 1994; Kasemuwan and Jane, 1994; McPherson and Jane, 1999). However, our attempts to analyze phosphorus in purified intact β-glucan samples were unsuccessful due to inherent problems of β-glucan in solution.

The major obstacle was the formation of highly viscous solutions even at concentration as low as 0.5% (w/w). Therefore, hydrolysis of β-glucan with lichenase enzyme was necessary. The hydrolysate contained smaller fragments of β-glucan having degree of polymerization (DP) of 3-9 with DP3 and DP4 being the predominant species based on high performance liquid chromatography (HPLC) and matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) analyses (Chapter 3). According to the information documented in the literature, phospholipids, phosphoproteins, inorganic phosphorus, pyrophosphates, and C-3 and C-6 inner carbon bound phosphate monoesters of hydrolyzed starches have chemical shifts of 0.2-0.3, 1.2-2.4 (Lim et al., 1994), 2.9, -6.6 (Lim and Seib, 1993), 1.71 and 1.01-1.15 ppm (Wood, 1993), respectively. ^{31}P NMR of concentrated hydrolysate of barley β-glucan showed three distinct types of phosphorus associations. Phosphorus with a chemical shift of 1.17 ppm is due to C-6 carbon bound phosphomonoester while the phosphorus with a chemical shift of –6.6 ppm was assigned to inorganic pyrophosphate (Fig. 4-2). The origin of pyrophosphate is unknown, but it is possible that pyrophosphate may have formed from the phosphate groups cleaved from phytates in barley β-glucan concentrate prepared using crude fungal and bacterial α-amylase and protease and co-precipitated during the β-glucan purification. Despite of its high phytate phosphorous content, in oat, the absence of pyrophosphate is surprising. The likely cause of de-phosphorylation of phytates was the employment of enzymes, low pH and heat during producing β-glucan concentrates. Similar to barley β-glucan hydrolysate, ^{31}P

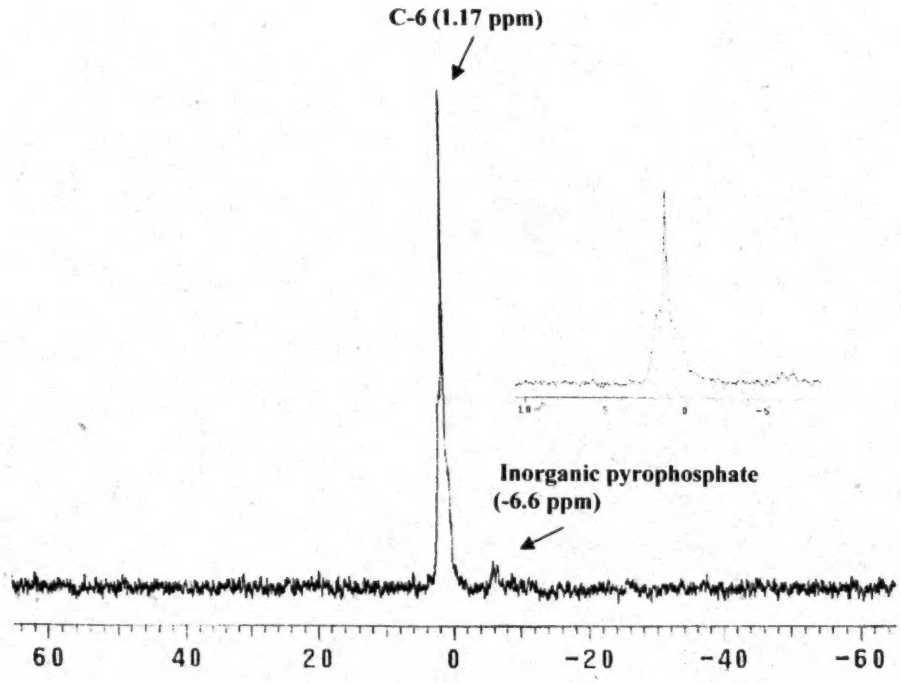

Figure 4-2 ^{31}P NMR spectrum of lichenase digested barley β-glucan.

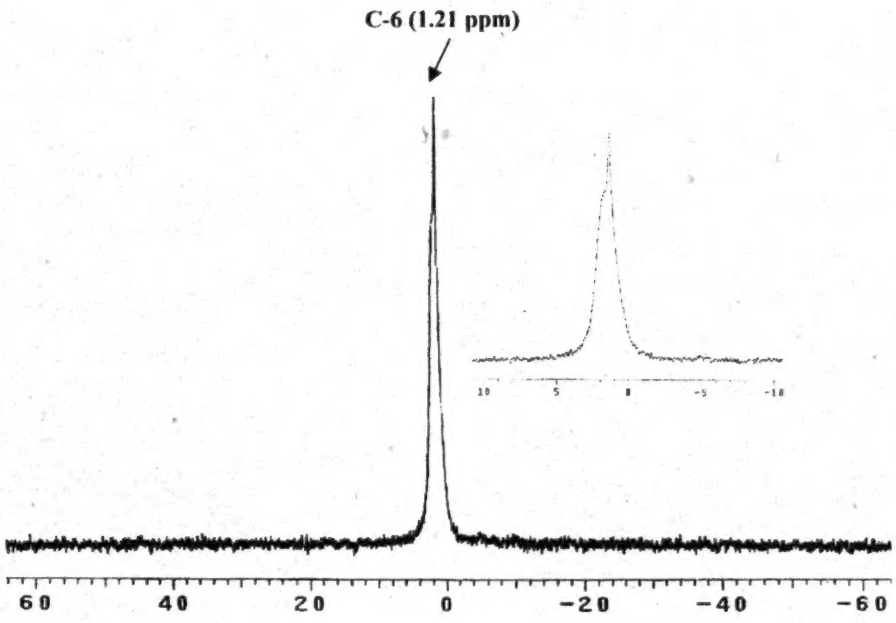

Figure 4-3 ^{31}P NMR spectrum of lichenase digested oat β-glucan.

NMR spectrum of oat β-glucan hydrolysate showed a signal at 1.21 ppm, which was assigned to C-6 carbon bound phosphomonoester (Figure 4-3). Pyrophosphate was not detected in oat β-glucan. Spectra of β-glucan from both sources showed a broad peak that overlapped with phosphomonoester signal in the region of 0 and 2 ppm and this may be due to the presence of some unknown forms of phosphorus, possibly phospholipids or phosphoproteins in oat β-glucan and the latter in barley β-glucan.

4.3.4 Solubility of β-glucan concentrates and laboratory purified β-glucans as related to phosphorus content and type

Solubility of dried crude and purified β-glucan preparations is dependent upon molecular weight, ratio of β-(1→3) and β-(1→4) linkages, method of drying and the presence of cross-linking agents, such as phytates (Beer et al., 1997; Kasemuwan and Jane, 1996; Beresford and Stone, 1983, McCleary, 1988; Woodward et al., 1988). Solubility of oat and barley β-glucan concentrates at 37°C (physiological solubility) was 28.9 and 3.6%, w/w, respectively. Oat β-glucan has been reported to contain a lower cellotriosyl:cellotetraosyl ratio than barley β-glucan (Cui et al., 2000). A lower proportion of cellotriosyl residue in β-glucan increases the non-uniformity in the cellulosic units (decreases the likelihood of ordered repeating of cellotriosyl residues), hence producing less ordered conformation (Cui et al., 2000). However, barley β-glucan, containing a higher proportion of cellotriosyl residue produces more ordered conformation that might be the cause of

its relatively poor solubility compared to oat β-glucan. Evidently, the relatively higher phytate content in oat β-glucan concentrate did not have a negative impact on its solubility. Solubility of purified oat β-glucan (73.7%, w/w, at 37 °C) was higher than that of purified barley β-glucan (11.1%, w/w at 37 °C), and is in good agreement with the solubility trends for their respective concentrates at the same temperature. This may partly be attributed to the high degree of phosphorus substitution (DS = 0.011) in oat β-glucan as opposed to the relatively low degree of substitution (DS = 0.005) in barley β-glucan. Oat β-glucan polymer chains with their high degree of negatively charged phosphate substitutions exerting a repulsive effect on similar charges of adjacent polymer chains have less opportunity to associate over the course of drying while this effect in barley β-glucan was not as strong as in oat due to the low degree of substitution. Therefore, the degree of phosphorylation coupled with the occurrence of β-(1→3) linkages in β-glucan polymer chains best explains the solubility characteristics of fiber materials used in this study.

4.4 Conclusions

Oat and barley β-glucan carry negatively charged phosphate groups in the form of phosphomonoesters bound to their C-6 carbons of inner anhydrous glucopyranosyl residues and thus, for the first time, the charged nature of the β-glucan polymer was established. In addition to molecular mass, ratio of β-(1→3) and β-(1→4) linkages and the drying method, the degree of substitution of the

negatively charged phosphate groups in the polymer also contribute to the solubility characteristics of β-glucan. The esterified phosphate groups on the β-glucan polymer chains exert repulsive forces on adjacent chains reducing the extent of aggregation during drying and enhancing hydration during re-dissolving.

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performance liquid chromatography of oligosaccharides released by lichenase. *Cereal Chem.*, 68: 31-39.

CHAPTER 5*
RHEOLOGICAL CHARACTERIZATION OF AQUEOUS SOLUTIONS CONTAINING β-GLUCAN AND GUMS

5.1 Introduction

β-Glucan, present in cell walls of barley and oat endosperm, has been known to possess physiological functionality showing demonstrated health benefits (Eastwood, 1992; Newman and Newman, 1992; Wood, 1993). In spite of this, barley remained an underutilized crop. Only 5% of barley produced in Canada is currently being utilized for direct human consumption. There is a potential of incorporating β-glucan into beverages and other food products thereby classifying β-as an glucan important functional food ingredient.

Due to functionality and cost considerations blends of food gums are often used in food formulations (Le Gloahec, 1951; Nnanna and Dawkins, 1996; Schorsch et al., 1997; Tako et al., 1998; Casas et al., 2000; Hernandez et al., 2001). An important parameter that determines the acceptability of gum blends in food and beverages is the stability of the blends throughout the product shelf life.

Studies directed towards the understanding of how barley β-glucan interacts with other food gums and the applicability of these interactions to foods and beverages are limited. Factors, such as the concentration of gum, temperature and pH of the medium, have a profound effect on the stability of β-glucan in solution

* A version of chapter 5 has been submitted for intellectual property designation (US patent application)

(Bansema, 2000). Moreover, the stability of gum mixtures in aqueous medium is also governed by the thermodynamic compatibility of gums constituting the system. Interactions between gums modify the rheological properties of gum mixtures and are important for new product development while improving the quality of the existing food products. For instance, the addition of *kappa*-carageenan to locust bean gum produces highly stable thermo-reversible gels with important synergistic effects (Tako et al., 1998). A mixture of gum arabic and carrageenan as an ice cream stabilizer has been patented (Le Gloahee, 1951) and it functions to retard both ice crystal formation and growth. Hence, the establishment of fundamental rheological properties of gum blends and the understanding of the interactions of barley β-glucan with other food gums are of importance. Therefore, this study was designed to provide insight into physical properties and functional properties of β-glucan in aqueous systems in combination with other food gums. The main objectives of the present study were:

1. To investigate the rheological properties of aqueous solutions of barley β-glucan (BBG) and binary gum blends consisting of BBG and commonly used food gums, namely xanthan (XAN), guar gum (GUA), locust bean gum (LBG), Konjac gum (KOG), low methoxyl pectin (LMP), high methoxyl pectin (HMP), gum arabic (GAR), carageenan (CAR) (*kappa*, *lamda*, and *iota*), sodium alginate (ALG), microcrystalline cellulose (MCC) and carboxymethyl cellulose (CMC),

2. To investigate the compatibility and aqueous phase stability of BBG and binary gum blends in terms of phase separation or precipitation observed visually over a period of 12 weeks at ambient temperature, and

3. To establish the most suitable gum blend containing β-glucan in terms of the product stability of a beverage system.

5.2 Materials and methods

5.2.1 Materials

A barley β-glucan concentrate (~60-65%, w/w, β-glucan) was obtained from Cevena BioProducts Inc. (Edmonton, AB). XAN was provided by ADM Inc. (Decatur, IL), whereas HMP, LMP, GUA, LBG, CMC and GAR were from TIC Gums Inc. (Belcamp, MD). KOG, MCC, CAR, and ALG were procured from FMC BioPolymer (Princeton NJ), while the crystallized beverage, Kool-Aid, was from Kraft Canada (North York, ON). Sodium carbonate, citric acid and hydrochloric acid were procured from BDH Inc. (Toronto, ON) and Fisher Scientific Co. (Nepean, ON), respectively. Ethanol and Termamyl 120 LN, a thermostable α-amylase (E.C. 3.2.1.1) of *Bacillus licheniformis*, were procured from Commercial Alcohols Inc. (Brampton, ON) and Novo Nordisk BioChem Inc. (Toronto, ON), respectively.

5.2.2 Extraction and purification of BBG from barley β-glucan concentrate

The purification of BBG from barley β-glucan concentrate was based on a traditional aqueous-alkali extraction methodology as shown in Figure 4-1. The method involved alkali extraction followed by enzymatic treatment. In brief, the

steps involved were the solubilization of BBG in deionized Milli-Q water, treatment with thermostable α-amylase (added at a rate of 1%, w/w, of available starch in the sample), followed by the protein denaturation and subsequent alcohol-assisted precipitation of BBG.

5.2.3 Chemical analyses

Contents of moisture, BBG, starch, and protein (N x 6.25) of dried samples was determined in duplicate according to the methods of McCleary and Glennie-Holmes (1985), Megazyme assay kit (Megazyme International Ireland Ltd., Ireland), Holm et al. (1986) and Hashimoto et al. (1987) and FP-428 Nitrogen Determinator (Leco Corp., St. Joseph, MI), respectively.

5.2.4 Determination of viscosity and thixotropy

Dispersions of BBG alone and its blends with common food gums (e.g. XAN, KOG, MCC, CAR, ALG, HMP, LMP, GUA, LBG, CMC and GAR) were prepared at a total gum concentration of 0.5% and 0.75% (w/w) in the ratios of 80/20 and 90/10 (w/w). For all binary blends, BBG was the major gum ingredient used. All gum solutions were prepared separately, heated at 90°C for 1 h and were allowed to cool down to room temperature. The gum blend dispersions were prepared by weighing and mixing at 80/20 and 90/10 (w/w) ratios of gum solutions prepared individually. The samples were then mixed for 20 min at room temperature to ensure uniform mixing.

Viscosity tests were performed for BBG and BBG binary blend dispersions. Viscosity was determined at consecutive fixed shear rates of 1.29-129 s^{-1} using a Paar Physica UDS 200 rheometer (Glenn, VA). The viscometer was equipped with a Peltier heating system that controlled the sample temperature. All viscosity tests were performed at 20°C using DG 27 cup and bob geometry with a 7±0.005 g sample. Shear rate was reported in s^{-1} after multiplying rpm by a conversion factor of 1.29 s^{-1} as specified by the manufacturer.

Thixotropy tests were also performed on both BBG and BBG binary blend dispersions using DG 27 cup and bob geometry with a 7±0.005 g sample at 20°C. These tests were performed at a series of fixed shear rates that consecutively increased from 1.29 to 3870 s^{-1} and then immediately decreased to the original shear rate of 1.29 s^{-1}. All analyses on gum blends were performed at least in duplicate.

5.2.5 Determination of viscoelastic properties of gum blends

All gum dispersions and gum blends were prepared using a similar procedure as described in sample preparation for viscosity and thixotropy analyses. Since the viscoelastic properties are strongly dependent on time and temperature, all systems were allowed to equilibrate for 15 min at ambient temperature. Storage modulus (G′) and loss modulus (G″) were obtained at 20°C using a 7±0.005 g sample placed in a DG 27 cup and bob geometry of a Paar Physica UDS 200 rheometer. The rheometer was set in amplitude sweep controlled shear displacement (CSD) mode with a constant frequency of 1 Hz and controlled strain of 0.25-20% and 0.75-120% for 0.5% and 0.75% total gum concentration, respectively.

5.2.6 Stability tests

The stability of BBG gum blends (at total gum concentrations of 0.5% and 0.75%, w/w, and gum ratios of 80/20 and 90/10, w/w) were compared with that of BBG dispersions alone. Sodium azide was added at 0.002% (w/w) to all samples to prevent microbial spoilage. Phase separation/precipitation was monitored subjectively by visual observation. The solutions were designated as "phase separated" when two distinct phases were visible. Stability was assessed subjectively by observing the gum blends for visible precipitation and phase separation over a period of 12 weeks at ambient temperature. Gum blends were evaluated on a scale of 1-4, where a score of 1 was assigned to solutions with extreme clarity with no visible precipitation while the extremely turbid solutions with extensive precipitation or phase separation were given a score of 4. All other situations were given either a score of 2 or 3, depending upon their visual characteristics.

5.2.7 Beverage formulation and evaluation of stability

The most compatible and stable gum blend, among gum combinations studied based on the observations made in the stability trials was selected for the beverage formulation. Two total gum concentrations selected were 0.23 and 0.46%, w/w. A concentration of 0.23% (w/w) was selected to reflect the minimum quantity of β-glucan per serving required for the health claim (0.75 g/serving) approved by FDA. XAN was added at a rate of 10% (w/w) of the amount of BBG present in order to achieve a final total gum concentration of 0.23% or 0.46% (w/w) and gum ratio of 90:10 (w/w). Eight grams of a crystallized commercial beverage (Kool-Aid)

were used for the preparation of 100 g of aqueous beverage containing gums at desired ratios. The final pH of the beverage was maintained at 3.25. Control beverage samples devoid of beverage crystals were prepared using gums and deionized Milli-Q water only. Two sets of control samples at pH 3.25 and 7 were prepared. Citric acid was used for adjusting the pH of control samples. All samples were stored at 4°C for 12 weeks.

The stability of beverage samples was assessed subjectively by observing any precipitation and changes in the viscosity over a storage period of 12 weeks at 4°C. Viscosity measurements were recorded using a Parr Physica UDS 200 rheometer (Glenn, VA). All timed viscosity measurements were taken at 5°C and 25°C (± 0.02° C) using DG 27 cup and bob geometry with a sample size of 7±0.005 g. Development of turbidity in the beverage was monitored spectrophotometrically at 660 nm (HP 8452A, Hewlett Packard, Boise, ID) (Bansema, 2000). To prevent the microbial spoilage over the storage period, sodium azide was added at 0.002% (w/w) to all beverage and control samples.

5.3 Statistical analysis

Purification experiments were performed in duplicates. All the chemical analyses were performed in duplicates for the purified concentrate fiber sample. Rheological measurements were performed for each of the purified samples in duplicates. Viscosity and phase stability tests for the gums or gum blends were also performed in duplicates for each of the purified samples.

5.4 Results and discussion

5.4.1 Recovery and composition of purified BBG

Recovery is defined as the ratio between the amount of BBG in purified sample and the amount of BBG present in barley β-glucan concentrate. The recovery and purity of purified BBG, obtained using the method given in Figure 5-1, were 82% and 94.7% (w/w, dry weight), respectively. Moisture, starch, and protein contents were 3.8%, 0.9% and 1.7% (w/w), respectively. Lipid content was 0% (w/w) in the barley β-glucan concentrate used and hence it was assumed that the purified barley β-glucan contains no lipids.

5.4.2 Viscosity of gum blends

In fluid flow behavior studies, the Power law model describes the pseudoplastic behavior of gums (Marcotte et al., 2001) (Eq. 2-1 in discussed in chapter 2). The flow behaviour index and consistency coefficient of 0.5% and 0.75% (w/w) pure gum dispersions are shown in Table 5-1. At 0.5% (w/w) concentration, HMP, LMP, ALG, *iota*-CAR, and GAR were almost Newtonian. However, at 0.75% (w/w) gum concentration, HMP and LMP continued to behave almost like Newtonian with $n \sim 0.99$, at a range of shear rate from 1.29 - 129 s^{-1}. BBG was highly pseudoplastic with a flow behavior index of 0.74 and 0.59 at 0.5% and 0.75% (w/w) concentrations, respectively. In comparison to other gums at 0.5% (w/w) concentration, XAN demonstrated high pseuodoplasticity with $n = 0.2$, followed by GUA with n = 0.38. In terms of flow behavior index, BBG at 0.5% (w/w) was comparable to CMC, LBG and KOG.

The viscosity of 0.5% and 0.75% (w/w) pure gums at 20°C determined at shear rates of 1.29-129 s^{-1}, is presented in Table 5-2. LMP, HMP, GAR, and MCC showed lower viscosity at both concentrations of 0.5% and 0.75% (w/w). The viscosity of all gum dispersions increased when the concentration was increased from 0.5 to 0.75% (w/w). The flow curves of individual gums and blends showed a shear thinning behavior, while yield stress was observed only in dispersions containing XAN, CAR and ALG. The yield stress that must be exceeded before the flow can begin was observed at lower shear rate. The concentration and shear rate effects on rheological properties were dependent upon the type of food gum used. The effect of concentration (0.5% and 0.75%, w/w) on viscosity enhancement was more pronounced in BBG, *iota*-CAR, and *kappa*-CAR dispersions as shown in Table 5-2. For XAN dispersions, however, the viscosity increased from 368 to 481 mPas at shear rate of 12.9 s^{-1} on increasing the gum concentration from 0.5% to 0.75% (w/w). This may be attributed to the near saturation of XAN dispersions at the concentrations tested. GUA, LBG and KOG dispersions demonstrated a better shear tolerance than other pure gum dispersions as evident by the viscosity data presented in Table 5-2. However, XAN demonstrated low shear rate tolerance at both gum concentrations tested in this study.

Blending of gums resulted in changes in certain rheological properties such as the viscosity, compared to the corresponding values for single components. The viscosities of gum blends having total gum concentrations of 0.5% and 0.75% (w/w), determined at shear rates of 1.29-129 s^{-1} at 20°C, are presented in Table 5-3.

Table 5-1 Flow behavior index (*n*) and consistency coefficient (*c*) at 0.5% and 0.75% (w/w) concentration of pure food gum dispersions determined at shear rates of 1.29–129 s^{-1} and a temperature of 20°C.

Pure gum systems	Flow behaviour index (*n*)	Consistency coefficient (*c*)	R^2
0.50% (w/w) gum concentration			
BBG	0.740	0.353	0.992
XAN	0.200	2.838	0.998
GUA	0.380	2.170	0.994
LBG	0.690	0.696	0.992
HMP	0.897	0.006	0.996
LMP	0.991	0.003	1.000
CMC	0.710	0.453	0.995
MCC	0.795	0.011	0.997
ALG	0.890	0.024	1.000
l-CAR	0.770	0.234	0.994
k-CAR	0.776	0.083	0.997
i-CAR	0.965	0.032	0.999
KOG	0.730	0.690	0.990
GAR	1.004	0.001	1.000
0.75% (w/w) gum concentration			
BBG	0.590	2.296	0.995
XAN	0.210	3.580	0.999
GUA	0.440	4.334	0.989
LBG	0.660	1.772	0.989
HMP	0.960	0.010	1.000
LMP	0.987	0.004	1.000
CMC	0.670	0.893	0.994
MCC	0.840	0.011	1.000
ALG	0.840	0.096	0.999
l-CAR	0.730	0.460	0.993
k-CAR	0.230	5.150	0.990
i-CAR	0.220	4.150	0.991
KOG	0.680	2.075	0.989
GAR	0.825	0.004	0.995

Values are means of duplicate determinations; BBG= barley beta-glucan; GUA= Guar gum; LBG=locust bean gum; HMP=high methoxl pectin; LMP=low methoxyl pectin; CMC=carboxymethyl cellulose; MCC=microcrystalline cellulose; ALG=alginate; *l*-CAR= *lambda*-carageenan; *k*-CAR= *kappa*-carageenan; *i*-CAR=*iota*-carageenan; KOG=Konjac; GAR= gum arabic

Table 5-2 Viscosity (mPa.s) of 0.5% and 0.75% (w/w) pure gum dispersions at shear rates of 1.29-129 s^{-1} and a temperature of 20°C.

Pure gums systems	Shear rate (1/s)					
	1.29	**6.46**	**12.9**	**25.8**	**64.6**	**129**
	0.5% (w/w) gum concentration					
BBG	287	237	203	166	118	87
XAN	2317	652	368	209	101	60
GUA	1193	667	466	310	172	108
LBG	394	360	327	279	200	144
HMP	6	4.2	3.9	3.8	3.7	3.7
LMP	4	4	4	4	3	3
CMC	378	283	235	189	135	101
MCC	12	7	6	6	5	4
ALG	24	20	18	17	16	78
l-CAR	196	166	146	123	92	70
k-CAR	71	59	51	43	32	25
i-CAR	31	30	30	29	28	26
KOG	550	455	389	316	221	159
GAR	1	1	1	1	1	1
	0.75% (w/w) gum concentration					
BBG	1890	1190	891	640	389	256
XAN	2908	834	481	277	132	78
GUA	3407	1693	1130	721	382	231
LBG	1447	1191	994	764	480	315
HMP	10	10	9	9	9	9
LMP	5.5	5.2	5.1	5.1	5.0	5.1
CMC	733	522	421	329	225	164
MCC	10	8	7	7	6	5
ALG	91	71	65	59	50	44
l-CAR	3317	1030	570	322	158	97
k-CAR	4043	1340	743	438	207	109
i-CAR	378	300	255	208	148	110
KOG	1720	1270	1020	768	489	326
GAR	4	3	2	2	2	2

Values are means of duplicate determinations; BBG= barley beta-glucan; GUA= Guar gum; LBG=locust bean gum; HMP=high methoxl pectin; LMP=low methoxyl pectin; CMC=carboxymethyl cellulose; MCC=microcrystalline cellulose; ALG=alginate; *l*-CAR= *lambda*-carageenan; *k*-CAR= *kappa*-carageenan; *i*-CAR=*iota*-carageenan; KOG=Konjac; GAR= gum arabic

Table 5-3 Viscosity (mPa.s) of 0.5% and 0.75% (w/w) BBG/other gum blend dispersions at shear rates of 1.29-129 s^{-1} and a temperature of 20°C.

Gum blend		Shear rate (1/s) 1.29	6.46	12.9	25.8	64.6	*n*	c
0.5% (w/w) gum concentration								
BBG/XAN	80/20	1277	540	378	261	158	0.46	1.47
	90/10	1090	531	390	278	174	0.51	1.29
BBG/GUA	80/20	408	308	252	196	131	0.65	0.56
	90/10	375	292	242	192	132	0.67	0.51
BBG/LBG	80/20	304	256	222	184	133	0.73	0.40
	90/10	324	264	226	184	130	0.71	0.43
BBG/HMP	80/20	151	134	120	103	79	0.87	0.21
	90/10	210	180	158	132	97	0.85	0.23
BBG/LMP	80/20	144	127	114	98	75	0.78	0.18
	90/10	155	136	121	103	79	0.77	0.19
BBG/CMC	80/20	763	493	381	284	182	0.58	1.02
	90/10	681	443	345	258	167	0.59	0.90
BBG/MCC	80/20	153	120	103	85	63	0.81	0.27
	90/10	200	163	140	116	84	0.73	0.35
BBG/ALG	80/20	232	192	166	139	102	0.74	0.29
	90/10	289	235	201	164	118	0.71	0.38
BBG/*l*-CAR	80/20	583	407	321	242	156	0.67	0.77
	90/10	506	358	285	216	141	0.67	0.80
BBG/*k*-CAR	80/20	219	183	158	130	94	0.69	0,38
	90/10	254	203	173	141	100	0.72	0.37
BBG/*i*-CAR	80/20	289	240	206	169	120	0.71	0.41
	90/10	314	256	217	175	123	0.69	0.43
BBG/KOG	80/20	276	232	200	165	119	0.70	0.45
	90/10	272	226	194	159	114	0.71	0.44
BBG/GAR	80/20	104	95	86	75	59	0.81	0.12
	90/10	176	152	134	113	84	0.76	0.22

Values are means of duplicate determinations.

n = flow behavior index; *c* = consistency coefficient; BBG= barley beta-glucan; GUA= Guar gum; LBG=locust bean gum; HMP=high methoxl pectin; LMP=low methoxyl pectin; CMC=carboxymethyl cellulose; MCC=microcrystalline cellulose; ALG=alginate; *l*-CAR= *lambda*-carageenan; *k*-CAR= *kappa*-carageenan; *i*-CAR=*iota*-carageenan; KOG=Konjac; GAR= gum arabic

Table 5-3...continued

Gum blend		Shear rate (1/s) 1.29	6.46	12.9	25.8	64.6	129	*n*	*c*
		0.75% (w/w) gum concentration							
BBG/XAN	80/20	3868	1634	1100	726	408	260	0.40	4.90
	90/10	4643	2049	1386	913	511	324	0.41	5.98
BBG/GUA	80/20	1870	1150	857	608	362	234	0.52	2.66
	90/10	1720	1100	830	598	363	239	0.54	2.45
BBG/LBG	80/20	1740	1160	891	651	399	262	0.55	2.59
	90/10	1797	1170	890	645	394	259	0.55	2.57
BBG/HMP	80/20	841	603	482	368	242	169	0.62	1.14
	90/10	1243	840	653	486	308	210	0.58	1.73
BBG/LMP	80/20	692	503	404	310	204	143	0.63	0.92
	90/10	1073	736	574	426	270	183	0.58	1.51
BBG/CMC	80/20	2607	1480	1074	751	444	290	0.49	3.61
	90/10	2580	1480	1076	752	444	290	0.50	3.58
BBG/MCC	80/20	1017	627	476	348	218	149	0.56	1.36
	90/10	1380	858	647	469	290	195	0.55	1.89
BBG/ALG	80/20	1193	788	610	454	290	200	0.58	1.62
	90/10	1413	920	706	519	326	220	0.57	1.95
BBG/*l*-CAR	80/20	1327	868	669	492	308	207	0.57	1.84
	90/10	1593	1020	779	566	349	231	0.55	2.24
BBG/*k*-CAR	80/20	1720	1030	768	550	334	221	0.53	2.37
	90/10	1827	1124	841	601	364	239	0.53	2.57
BBG/*i*-CAR	80/20	2323	1370	1000	697	402	255	0.49	3.36
	90/10	2217	1320	970	681	400	257	0.50	3.16
BBG/KOG	80/20	1733	1140	874	638	394	261	0.56	2.46
	90/10	1840	1180	895	648	397	262	0.54	2.61
BBG/GAR	80/20	625	465	377	290	192	135	0.64	0.84
	90/10	1033	709	554	413	262	178	0.60	1.40

Values are means of duplicate determinations.
n = flow behavior index; *c* = consistency coefficient; BBG= barley beta-glucan; GUA= Guar gum; LBG=locust bean gum; HMP=high methoxl pectin; LMP=low methoxyl pectin; CMC=carboxymethyl cellulose; MCC=microcrystalline cellulose; ALG=alginate; *l*-CAR= *lambda*-carageenan; *k*-CAR= *kappa*-carageenan; *i*-CAR=*iota*-carageenan; KOG=konjac; GAR= gum arabic

At 0.5% (w/w) total gum concentration, BBG blend with XAN, CMC and *lambda*-CAR showed marked enhancement in viscosity, while BBG blend with KOG, HMP, LMP, ALG, MCC and GAR showed marked lowering of viscosity compared to the BBG alone. At 0.75% (w/w) total gum concentration, BBG blend with XAN, *iota*-CAR, and CMC showed marked viscosity enhancement compared to BBG alone. However, BBG blend with *lambda*-CAR, KOG, HMP, LMP, MCC, ALG, and GAR gum showed marked lowering of the viscosity than compared to BBG alone.

As shown in Table 5-2, at a shear rate of 64.6 s^{-1}, 0.5% (w/w) BBG and XAN individually exhibited viscosities of 118 and 101 mPas, respectively, whereas in Table 5-3, 0.5% (w/w) BBG/XAN blended in 80/20 and 90/10 (w/w) ratios demonstrated viscosities of 158 and 174 mPas, respectively. Thus, the BBG/XAN blend was more shear tolerant as evident by the floe behavior index value (Table 5-3) than BBG or XAN alone. Similar trends were also observed with BBG/CMC and BBG/*lambda*-CAR at low concentrations (i.e. 0.5%, w/w) and also with BBG/CMC and BBG/*iota*-CAR at higher concentrations (i.e. 0.75%, w/w).

Synergistic interactions between gums in a blend are governed by hydrogen bonding (Bresolin et al., 1998). The viscosity synergism observed in this study between XAN (branched) and BBG (linear) gum blend may be attributed to their unique molecular structure/conformation. We postulate that the linear BBG molecules could become entangled within the branches of XAN molecules, satirically interfering with the intermolecular associations among the cellulosic

segments of BBG strands thus preventing/suppressing phase separation and thereby, improving phase stability of a solution. In addition, the formation of hydrogen bonds among hydroxyl groups of XAN and BBG molecules would enhance the stability of the entanglements, improving shear tolerance (less pseudoplastic) and the apparent viscosity of the solution.

The total gum concentration and ratio of gums in a blend affect the rate and the type of interaction (synergistic or antagonistic) as demonstrated by the viscosity measurements. These interactions were quantified using "viscous synergism index", I_v, (Eq. 2-3) discussed in detail in chapter 2.

Tables 5-4 and 5-5 show the "viscous synergism index", I_v calculated for 0.5% and 0.75% (w/w) BBG/other gum blends, respectively, using the viscosity data determined at a shear rate of 6.46 s^{-1} (to mimic the approximate shear that exists in human mouth) at 20°C. For gum blends such as BBG/CMC, BBG/*lambda*-CAR and *iota*-CAR at 0.5% (w/w) total concentration, at both 80/20 and 90/10 (w/w) blending ratios, synergistic interactions were observed. However, other gum blends at 0.5% (w/w) total concentration such as BBG/XAN, BBG/GUA, BBG/LBG, BBG/HMP, BBG/LMP, BBG/*kappa*-CAR, BBG/ALG, BBG/GAR, BBG/MCC, and BBG/KOG demonstrated antagonistic interactions at both 80/20 and 90/10 (w/w) blending ratios. For gum blends at 0.75% (w/w) total concentration, synergistic interactions were observed in the blends of BBG with XAN, CMC and *iota*-CAR at both 80/20 and 90/10 (w/w) blending ratios. However, blending of BBG with LBG at 0.75% (w/w) total gum concentration at both 80/20 and 90/10 (w/w) blending ratios was

Table 5-4 Viscous synergism index, I_v, of 0.5% (w/w) BBG/other gum blend dispersions at a shear rate of 6.46 s^{-1} and a temperature of 20°C.

	Viscosity at 6.46 s^{-1}					
Gum blend	**η (i)**	**η (j)**	**η (i) +η (j)**	**η (i+j)**	**I_v**	**Interaction**
			Blend ratio 80/20 (w/w)			
BBG/XAN	237	652	889	540	0.61	antagonism
BBG/GUA	237	667	904	308	0.34	antagonism
BBG/LBG	237	360	597	256	0.43	antagonism
BBG/HMP	237	4.2	241.2	134	0.56	antagonism
BBG/LMP	237	3.5	240.5	127	0.53	antagonism
BBG/CMC	237	283	520	493	0.95	synergism
BBG/MCC	237	7	244	120	0.49	antagonism
BBG/ALG	237	20	257	192	0.75	antagonism
BBG/*l*-CAR	237	166	403	407	1.01	synergism
BBG/*k*-CAR	237	59	296	183	0.62	antagonism
BBG/*i*-CAR	237	30	267	240	0.90	synergism
BBG/KOG	237	455	692	232	0.34	antagonism
BBG/GAR	237	1.1	238.1	95	0.40	antagonism
			Blend ratio 90/10 (w/w)			
BBG/XAN	237	652	889	531	0.60	antagonism
BBG/GUA	237	667	904	292	0.32	antagonism
BBG/LBG	237	360	597	264	0.44	antagonism
BBG/HMP	237	4.2	241.2	180	0.75	antagonism
BBG/LMP	237	3.5	240.5	136	0.57	antagonism
BBG/CMC	237	283	520	443	0.85	synergism
BBG/MCC	237	7	244	163	0.67	antagonism
BBG/ALG	237	20	257	235	0.91	antagonism
BBG/*l*-CAR	237	166	403	358	0.89	synergism
BBG/*k*-CAR	237	59	296	203	0.69	antagonism
BBG/*i*-CAR	237	30	267	256	0.96	synergism
BBG/KOG	237	455	692	226	0.33	antagonism
BBG/GAR	237	1.1	238.1	152	0.64	antagonism

Values are means of duplicate determinations. All viscosity measurements [η(i), (η(j) and η (i+j)] were performed at identical total gum concentration (0.5%, w/w). BBG= barley beta-glucan; GUA= Guar gum; LBG=locust bean gum; HMP=high methoxl pectin; LMP=low methoxyl pectin; CMC=carboxymethyl cellulose; MCC=microcrystalline cellulose; ALG=alginate; *l*-CAR= *lambda*-carageenan; *k*-CAR= *kappa*-carageenan; *i*-CAR=*iota*-carageenan; KOG=Konjac; GAR= gum arabic

Table 5-5 Viscous synergism index, I_v, of 0.75% (w/w) BBG/other gum blend dispersions at a shear rate of 6.46 s^{-1} and a temperature of 20°C

	Viscosity at 6.46 s^{-1}					
Gum blend	**η (i)**	**η (j)**	**η (i) + η (j)**	**η (i+j)**	**I_v**	**Interaction**
			Blend ratio 80/20 (w/w)			
BBG/XAN	1190	834	2024	1634	0.81	synergism
BBG/GUA	1190	1693	2883	1150	0.40	antagonism
BBG/LBG	1190	1191	2381	1160	0.49	no interaction**
BBG/HMP	1190	10	1199	603	0.50	antagonism
BBG/LMP	1190	5	1195	503	0.42	antagonism
BBG/CMC	1190	522	1712	1480	0.86	synergism
BBG/MCC	1190	8.1	1198	627	0.52	antagonism
BBG/ALG	1190	71	1261	788	0.62	antagonism
BBG/*l*-CAR	1190	1030	2220	868	0.39	antagonism
BBG/*k*-CAR	1190	1340	2530	1030	0.41	antagonism
BBG/*i*-CAR	1190	300	1490	1370	0.92	synergism
BBG/KOG	1190	1270	2460	1140	0.46	antagonism
BBG/GAR	1190	3	1192	465	0.39	antagonism
			Blend ratio 90/10 (w/w)			
BBG/XAN	1190	834	2024	2049	1.01	synergism
BBG/GUA	1190	1693	2883	1100	0.38	antagonism
BBG/LBG	1190	1191	2381	1170	0.49	no interaction**
BBG/HMP	1190	10	1199	840	0.70	antagonism
BBG/LMP	1190	5	1195	736	0.61	antagonism
BBG/CMC	1190	522	1712	1480	0.86	synergism
BBG/MCC	1190	8	1198	858	0.71	antagonism
BBG/ALG	1190	71	1261	920	0.72	antagonism
BBG/*l*-CAR	1190	1030	2220	1020	0.46	antagonism
BBG/*k*-CAR	1190	1340	2530	1124	0.44	antagonism
BBG/*i*-CAR	1190	300	1490	1320	0.88	synergism
BBG/KOG	1190	1270	2460	1180	0.48	antagonism
BBG/GAR	1190	3	1192	709	0.59	antagonism

** Marginally antagonistic; Values are means of duplicate determinations. All viscosity measurements [η(i), (η(j) and η (i+j)] were performed at identical total gum concentration (0.75%, w/w); BBG= barley beta-glucan; GUA= Guar gum; LBG=locust bean gum; HMP=high methoxl pectin; LMP=low methoxyl pectin; CMC=carboxymethyl cellulose; MCC=microcrystalline cellulose; ALG=alginate; *l*-CAR= *lambda*-carageenan; *k*-CAR= *kappa*-carageenan; *i*-CAR=*iota*-carageenan; KOG=konjac; GAR= gum arabic

designated as "no interaction" as the viscosities of the resulting blends was similar to the viscosity of the individual gums. Furthermore, an antagonistic effect was observed for the gum blends at 0.75% (w/w) total concentration at both 80/20 and 90/10 (w/w) blending ratios when BBG was blended with GUA, HMP, LMP, ALG, KOG, MCC, *lambda*-CAR and GAR. *lambda*-CAR behaved synergistically when mixed with BBG to achieve total concentration of 0.5% (w/w), whereas at 0.75% (w/w) total concentration, these gums demonstrated strong antagonism. In BBG/XAN blends (80/20 and 90/10, w/w), an antagonistic effect was observed at 0.5% (w/w) total gum concentration. The effect transformed into strong synergism with $I_v = 0.8$ when total gum concentration was increased to 0.75% (w/w). Unlike the blends having 0.5% (w/w) total gum concentration, the blends of BBG/LBG at 0.75% (w/w) total concentration showed no interaction at both ratios tested.

5.4.3 Thixotropy of gum blends

The phenomenon of thixotropy was originally introduced to define an isothermal sol ↔ gel transformation (Freundlich, 1935; Sherman, 1970). Thixotropy can be defined as a decrease in viscosity due to destruction of structure under a constant shear rate or a consecutively increasing shear rate that is fixed for a period of time at selected shear rates followed by the structural network redevelopment when shear is withdrawn (Muller, 1973; Schramm, 1994). The viscosity of non-thixotropic systems does not decrease with time when exposed to constant shear rates. Under consecutively increasing shear rates the viscosity decreases, but regains over time when shear is withdrawn.

In the present study, the thixotropy was examined, using consecutive increasing shear rates of 1.29-3870 s^{-1} for fixed intervals of time and then decreasing it immediately to the original shear rate of 1.29 s^{-1}. Figure 5-1 shows non-thixotropic behaviour observed for 0.5% and 0.75% (w/w) BBG dispersions. Autio et al. (1987) also reported a similar behavior for β-glucan dispersions. Figure 5-2 and Figure 5-3 depict the thixotropy curves at 20°C of 0.5% and 0.75% (w/w) BBG/other gum blends, respectively. None of the gum blends used in the study demonstrated thixotropy. For pure BBG dispersions, the time required for the viscosity to redevelop at 1.29 s^{-1} exceeded 4-6 min. However, 0.5% (w/w) BBG/MCC blend showed inability of viscosity redevelopment at 1.29 s^{-1} after being exposed to high shear (3870 s^{-1}), hence showing thixotropy. BBG/XAN blended at a ratio of 80/20 (w/w) at 0.5 and 0.75% (w/w) total gum concentrations recovered its original viscosity in 10-15 sec. Interestingly, during the thixotropy testing, 80/20 and 90/10 (w/w) BBG/XAN blends demonstrated unusual increase in viscosity upon immediately decreasing the shear rate from 3870 s^{-1} to 1.29 s^{-1} compared to the original viscosity at the starting shear rate of 1.29 s^{-1}. This shear-induced thickening of the blend dispersion suggested a change in polymer conformation. Change in XAN conformations in aqueous medium has been reported elsewhere, but the change occurred due to heating (Kovacs and Kang, 1977; Bresolin et al., 1998). In the present study, the shear rate of 3870 s^{-1} employed during thixotropy testing might have resulted in unwinding of the ordered helical conformation of XAN into disordered

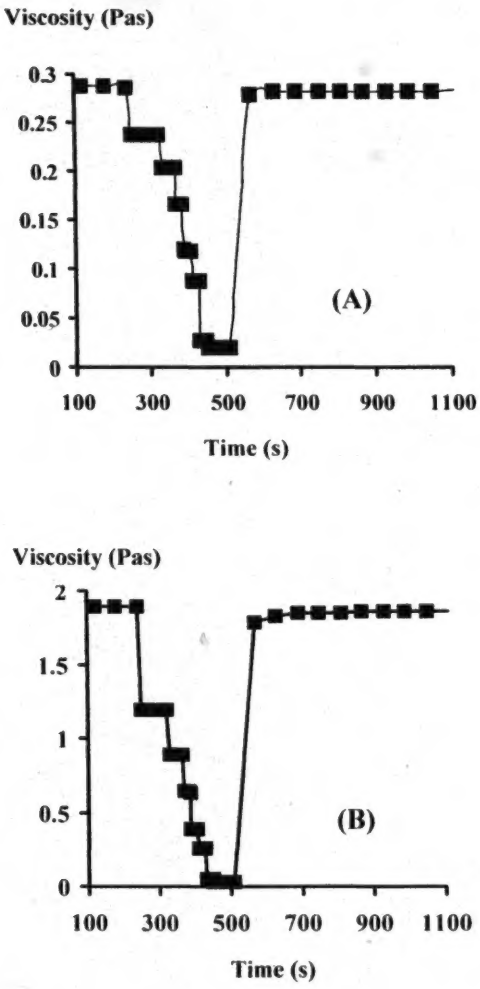


Figure 5-1 Thixotropy curves of purified BBG determined at shear rates of 1.29-3870 s^{-1} at 20°C. (A) BBG at 0.5% (w/w), (B) BBG at 0.75% (w/w).
(BBG = barley beta-glucan)

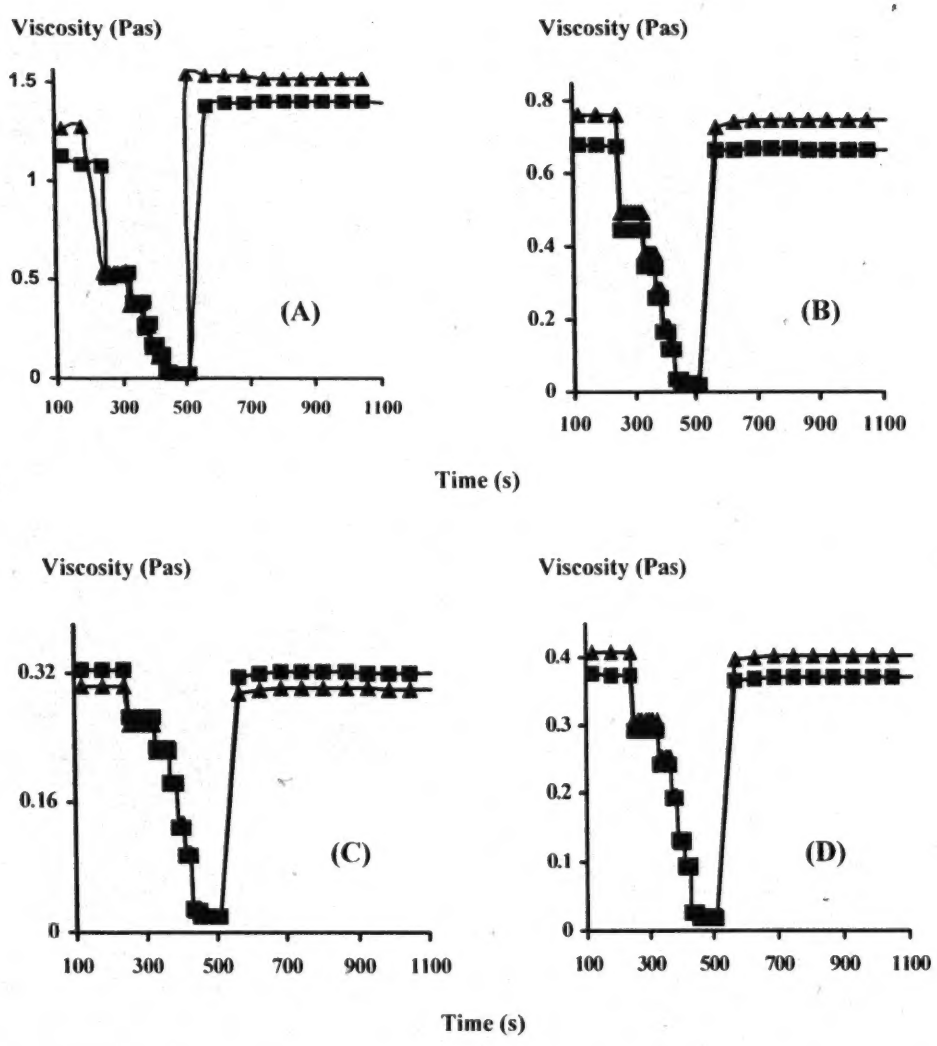


Figure 5-2 Thixotropy curves of 0.5% (w/w) BBG/other gum blends after shearing at 3870 s^{-1} at 20°C. (■) BBG/other gum ratio of 90/10, w/w, (▲) BBG/other gum ratio of 80/20, w/w.
(A) BBG/XAN, (B) BBG/CMC, (C) BBG/LBG blend,
(D) BBG/GUA,
(BBG= barley beta-glucan; GUA= Guar gum; LBG=locust bean gum; CMC=carboxymethyl cellulose)

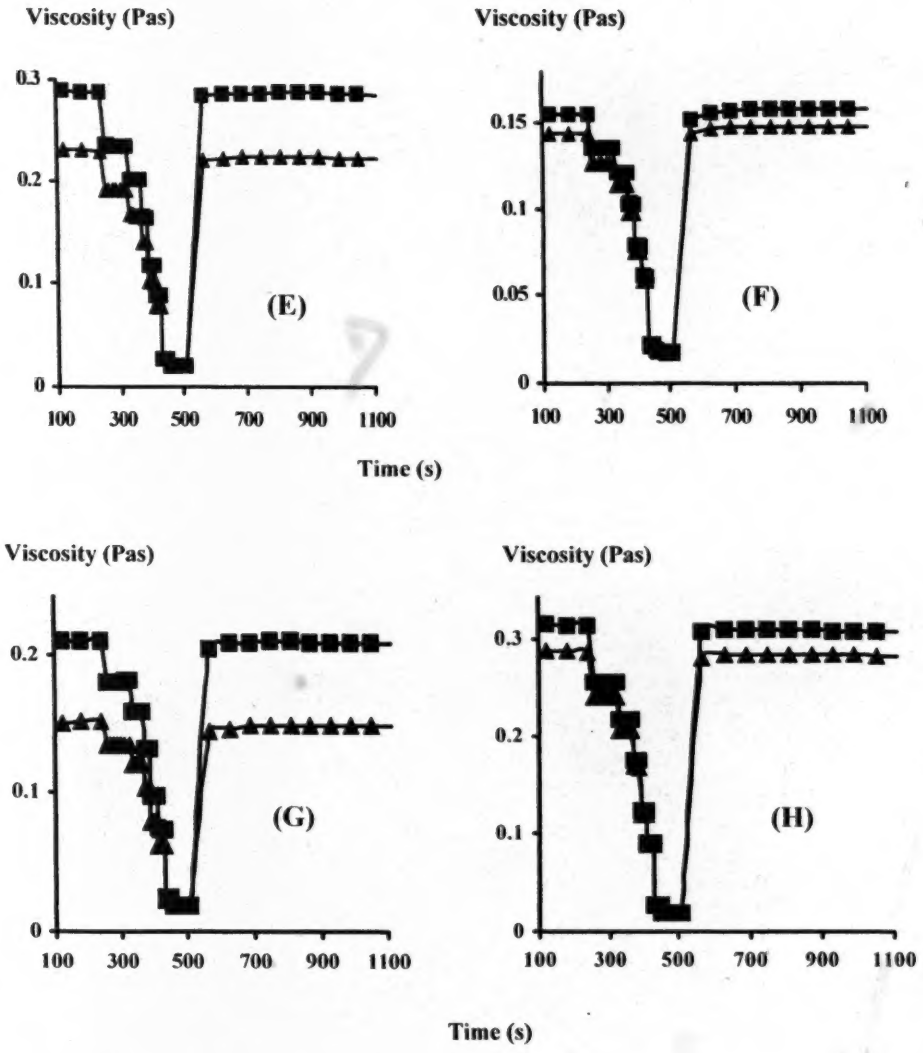


Figure 5-2 Thixotropy curves of 0.5% (w/w) BBG/other gum blends after shearing at 3870 s^{-1} at 20°C. (■) BBG/other gum ratio of 90/10, w/w, (▲) BBG/other gum ratio of 80/20, w/w.

(E) BBG/ALG, (F) BBG/LMP, (G) BBG/HMP,
(H) BBG/*i*-CAR
(BBG= barley beta-glucan; HMP=high methoxl pectin; LMP=low methoxyl pectin; *i*-CAR=*iota*-carageenan; ALG=alginate)

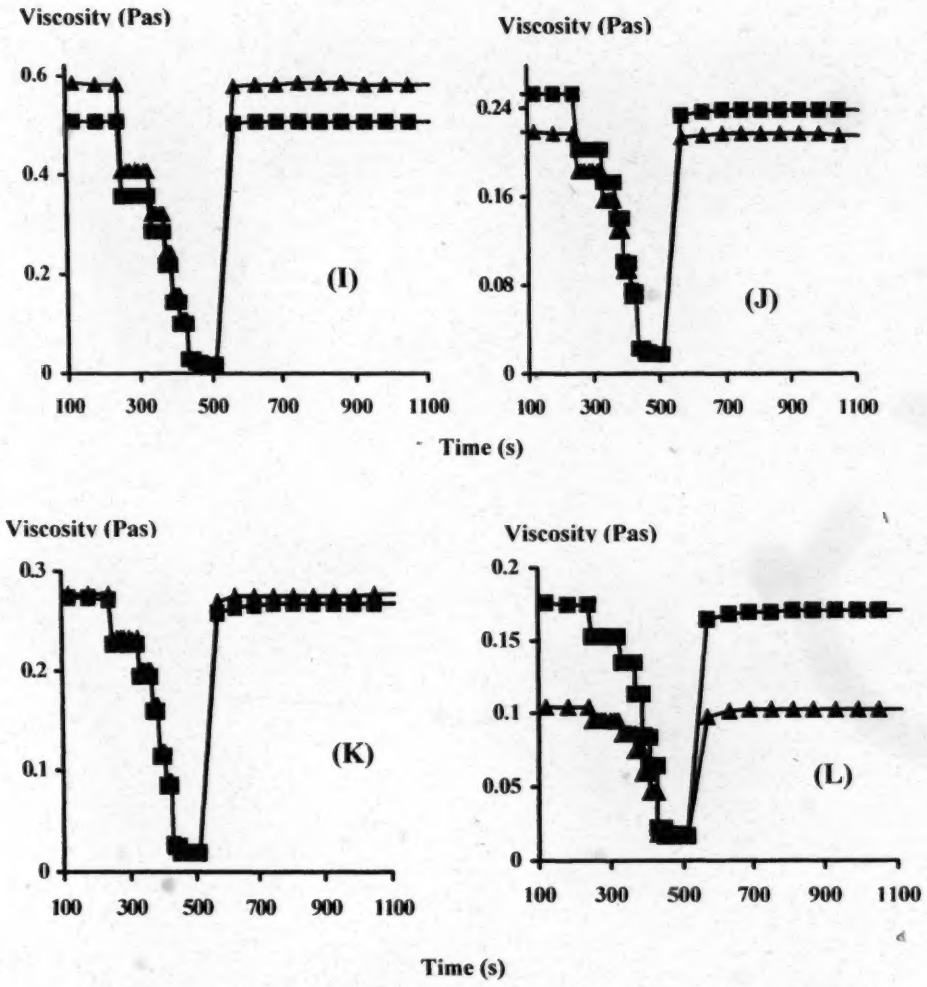


Figure 5-2 Thixotropy curves of 0.5% (w/w) BBG/other gum blends after shearing at 3870 s^{-1} at 20°C. (■) BBG/other gum ratio of 90/10, w/w, (▲) BBG/other gum ratio of 80/20, w/w.

(I) BBG/*l*-CAR, (J) BBG/*k*-CAR, (K) BBG/KOG, (L) BBG/GAR

(BBG= barley beta-glucan; *l*-CAR= *lambda*-carageenan; *k*-CAR= *kappa*-carageenan; KOG=Konjac; GAR= gum arabic)

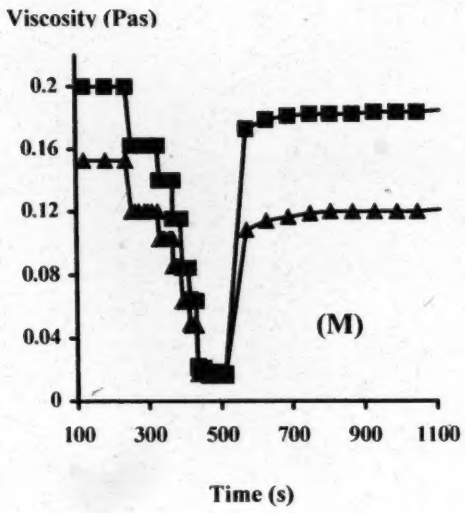


Figure 5-2 Thixotropy curves of 0.5% (w/w) BBG/other gum blends after shearing at 3870 s^{-1} at 20°C. (■) BBG/other gum ratio of 90/10, w/w, (▲) BBG/other gum ratio of 80/20, w/w. (M) BBG/MCC

(BBG= barley beta-glucan; MCC=microcrystalline cellulose)

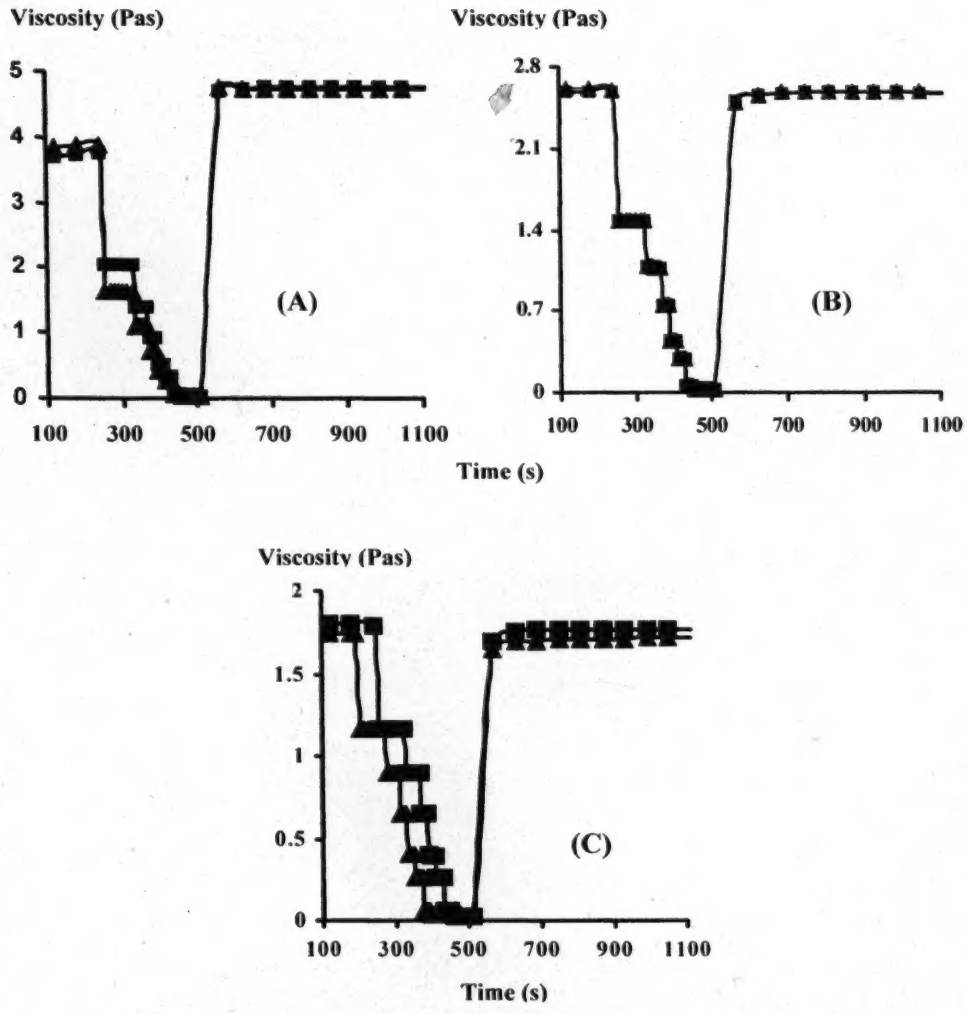


Figure 5-3 Thixotropy curves of 0.75% (w/w) purified BBG after shearing at 3870 s^{-1} at 20°C (■) BBG/other gum ratio of 90/10, w/w, (▲) BBG/other gum ratio of 80/20, w/w.
(A) BBG/XAN, (B) BBG/CMC, (C) BBG/LBG blend
(BBG= barley beta-glucan; XAN= xanthan; LBG=locust bean gum; CMC=carboxymethyl cellulose;

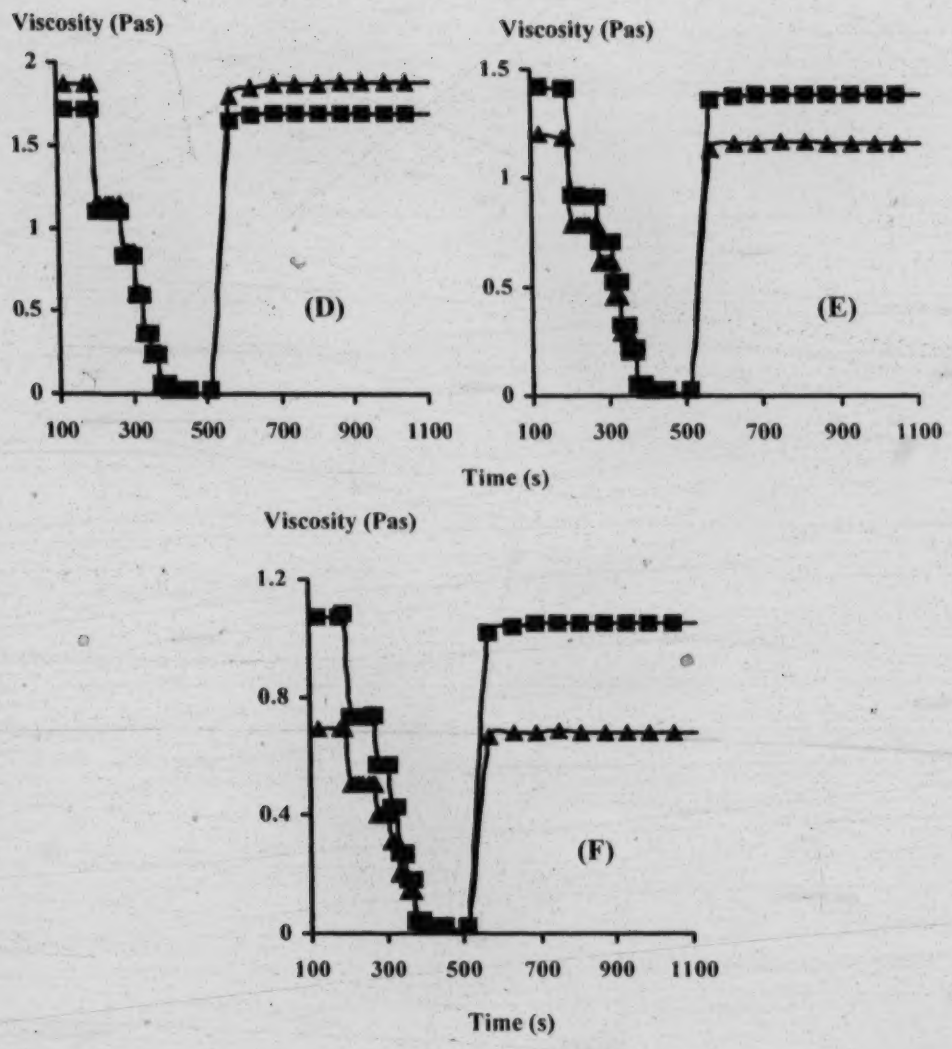


Figure 5-3 Thixotropy curves of 0.75% (w/w) purified BBG after shearing at 3870 s^{-1} at 20°C (■) BBG/other gum ratio of 90/10, w/w, (▲) BBG/other gum ratio of 80/20, w/w.
(D) BBG/GUA, (E) BBG/ALG,-(F) BBG/LMP
(BBG= barley beta-glucan; GUA= Guar gum; LMP=low methoxyl pectin; ALG=alginate)

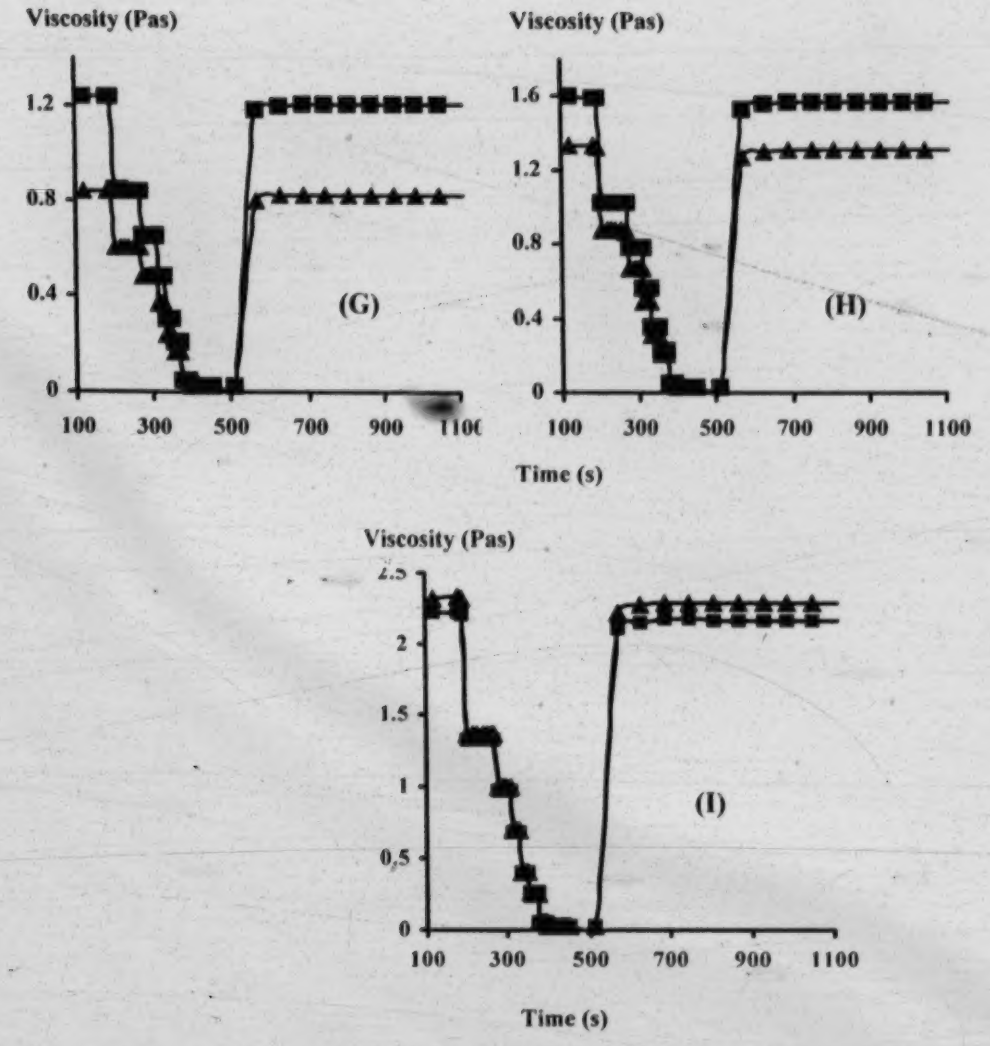


Figure 5-3 Thixotropy curves of 0.75% (w/w) purified BBG after shearing at 3870 s^{-1} at 20°C (■) BBG/other gum ratio of 90/10, w/w, (▲) BBG/other gum ratio of 80/20, w/w.
(G) BBG/HMP, (H) BBG/*i*-CAR, (I) BBG/*lambda*-CAR
(BBG= barley beta-glucan; HMP=high methoxl pectin; *l*-CAR= *lambda*-carageenan; *i*-CAR=*iota*-carageenan;

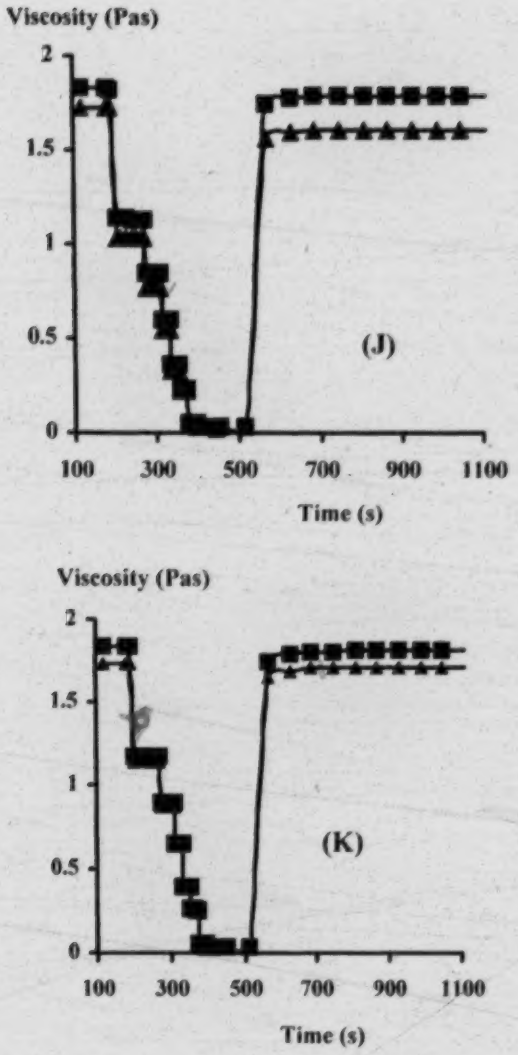


Figure 5-3 Thixotropy curves of 0.75% (w/w) purified BBG after shearing at 3870 s^{-1} at 20°C (■) BBG/other gum ratio of 90/10, w/w, (▲) BBG/other gum ratio of 80/20, w/w.
(J) BBG/*k*-CAR, (K) BBG/KOG
(BBG= barley beta-glucan; *k*-CAR= *kappa*-carageenan; KOG=Konjac)

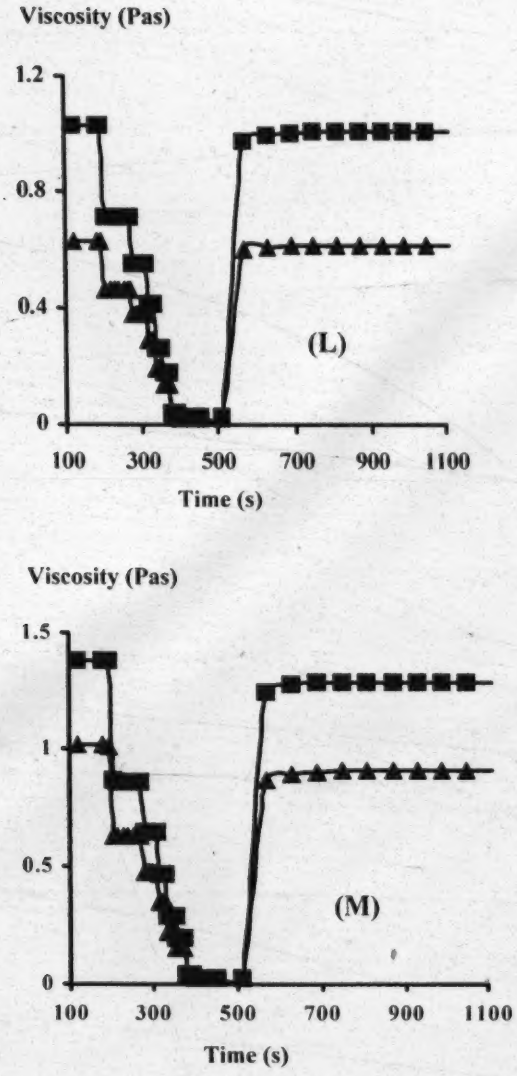


Figure 5-3 Thixotropy curves of 0.75% (w/w) purified BBG after shearing at 3870 s^{-1} at 20°C (■) BBG/other gum ratio of 90/10, w/w, (▲) BBG/other gum ratio of 80/20, w/w.
(L) BBG/GAR, (M) BBG/MCC
(BBG= barley beta-glucan; MCC=microcrystalline; GAR= gum arabic)

random coil conformation, a cellulose-like conformation, and thus increasing the hydrodynamic volume and hence the increased viscosity.

5.4.4 Elastic modulus of gum blends

Storage modulus (G′) and loss modulus (G″) define the viscoelastic properties of gum solutions (Mandala and Palogou, 2003; Skendi et al., 2003). G′ and G″ at controlled strain and constant frequency (1 Hz) were recorded in order to locate the linear viscoelastic region (Dickinson and Merino, 2002; Mandala and Palogou, 2003). A typical curve of G′ and G″ values versus strain defining a linear viscoleastic region has been discussed elsewhere (Mandala and Palogou, 2003). Deviations from linearity occur when the gel is strained to a point at which certain weak physical bonds of the aggregated network structure are destroyed. Formation of new bonds will also influence the linear viscoelastic region. In general, gels have much shorter linear regions than cross-linked polymer gels (Dickinson and Merino, 2002).

In the present study, an amplitude sweep was applied where stress and strain were increased linearly at a constant frequency of 1 Hz. Dependence of G′ and G″ on frequencysweep was not performed in the present study because it was beyond the scope of the present study. Frequency sweep is important to determine the time required for polymer entanglements to form or break within the variable periods of oscillations (Lazaridou et al., 2003). A constant frequency of 1Hz was selected to allow sufficient time for network (polymer entanglements) to form and break

because at higher frequencies, the molecular chains cannot disentangle during the short periods of oscillation (Lazaridou et al., 2003).

A gel-like material shows distinct behavior that is different from liquid or concentrated solution when subjected to amplitude sweep in a rheometer at constant frequency. Freshly prepared BBG dispersions have been reported to behave like a viscoelastic liquid ($G'' > G'$) where the G' and G'' are reported to be highly dependent on frequency (Skendi et al., 2003). Formation of an elastic gel-like network ($G' > G''$) depends on the gum concentration as well as the induction time of gelation. Once the gel like viscoleastic properties are gained, the G' and G'' become less dependent on frequency (Lazaridou et al., 2003).

Comparison of G' and G'' for 0.5 and 0.75% (w/w) BBG dispersions was performed at linearly increasing strain of 0.25-20% and 0.75-120%, respectively at a constant frequency of 1 Hz. For 0.5% (w/w) gum dispersions, the ramp of strain was carefully selected to ensure that the stress used was not exceeding 1 Pa. A strain range of 0.25-20% was selected based on observations for preliminary experiments with 0.5% (w/w) gum dispersions and blends at different levels of strain sweep in order to prevent the destruction of physical bonds that contribute to the elastic properties. However, for 0.75% (w/w) gum dispersions and their blends, strain sweep of 0.075-120% was selected to ensure the stress used was not exceeding 10 Pa. The main reason for selecting a maximum stress of 1 Pa for 0.5% (w/w) and 10 Pa for 0.75% (w/w) gum and gum blend dispersions was to enable the comparison of linear viscoelastic regions of different BBG/other gum blends to that of pure BBG

dispersions. Figure 5-4 shows comparison of G′ and G″ for 0.5 and 0.75% (w/w) BBG dispersions determined at 20°C. Both 0.5% and 0.75% (w/w) BBG dispersions demonstrated viscoelastic behavior since G″ > G′. This is in agreement with other viscoleastic studies of oat and barley β-glucan dispersions of different concentrations (Lazaridou et al., 2003). Figure 5-5 presents comparison of G′ and G″ for 0.5% BBG/other gum blends. Both gum ratios of 80/20 and 90/10 (w/w) of 0.5% (w/w) BBG/GUA, BBG/LBG, BBG/CMC, BBG/CAR, and BBG/KOG blends exhibited viscoelastic behaviour with G″ > G′ (Figure 5-5). However, 0.5% (w/w) BBG/XAN blend mixed at a ratio of 80/20 (w/w) became typical of an elastic gel network with G′ > G″. Such an elastic gel like behavior was not exhibited by 90/10 (w/w) BBG/XAN blends at 0.5% (w/w) total gum concentration. Hence, BBG/XAN ratio of 80/20 (w/w) mixed at 0.5% (w/w) total gum concentration is critical for the development of a gel-like behavior (Fig. 5-3(A)). Elastic network formation may be the reason for faster recovery time observed soon after the network destruction at 3870 s^{-1} during thixotropy testing. G′ and G″ values decreased as the proportion of XAN increased from 10-20% (w/w) in 0.5% (w/w) BBG/XAN blend. Blends containing BBG and HMP, LMP, *iota*-CAR, MCC, ALG and GAR, having a total

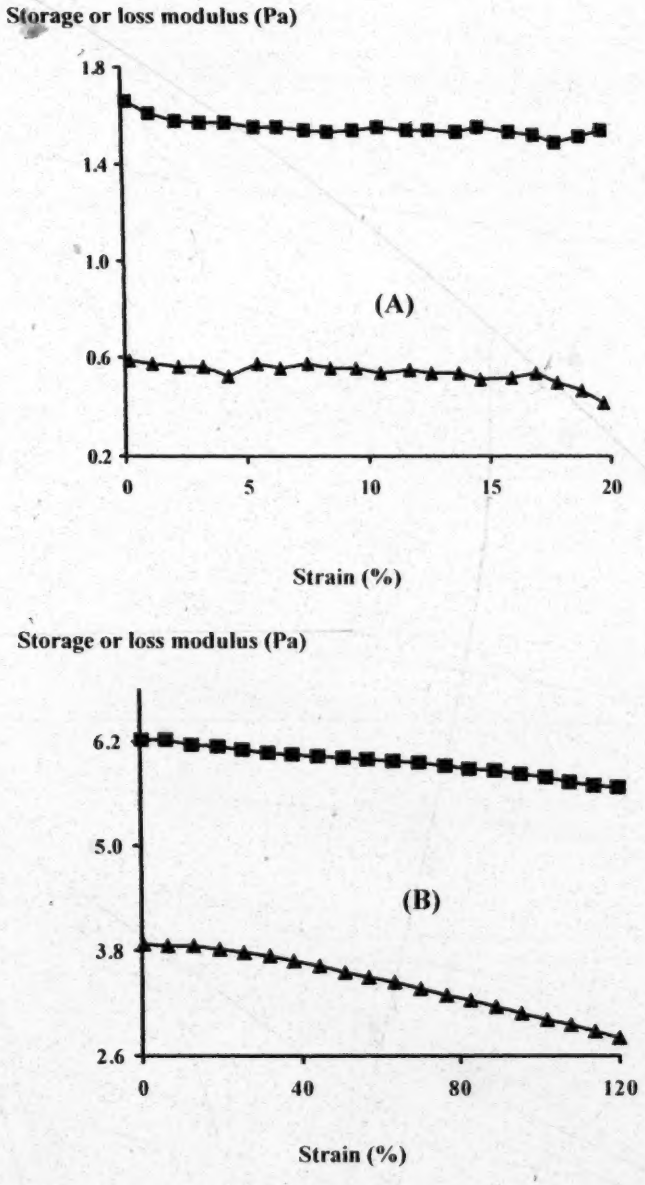


Figure 5-4 Graphs showing a comparison of (▲) storage modulus (G′) and (■) loss modulus (G″) of barley beta-glucan (BBG) solution at 20°C. (A) 0.5% (w/w) BBG determined at 0.075-20% strain and 1 Hz frequency, (B) 0.75% (w/w) BBG determined at 0.25%-120% strain and 1 Hz frequency

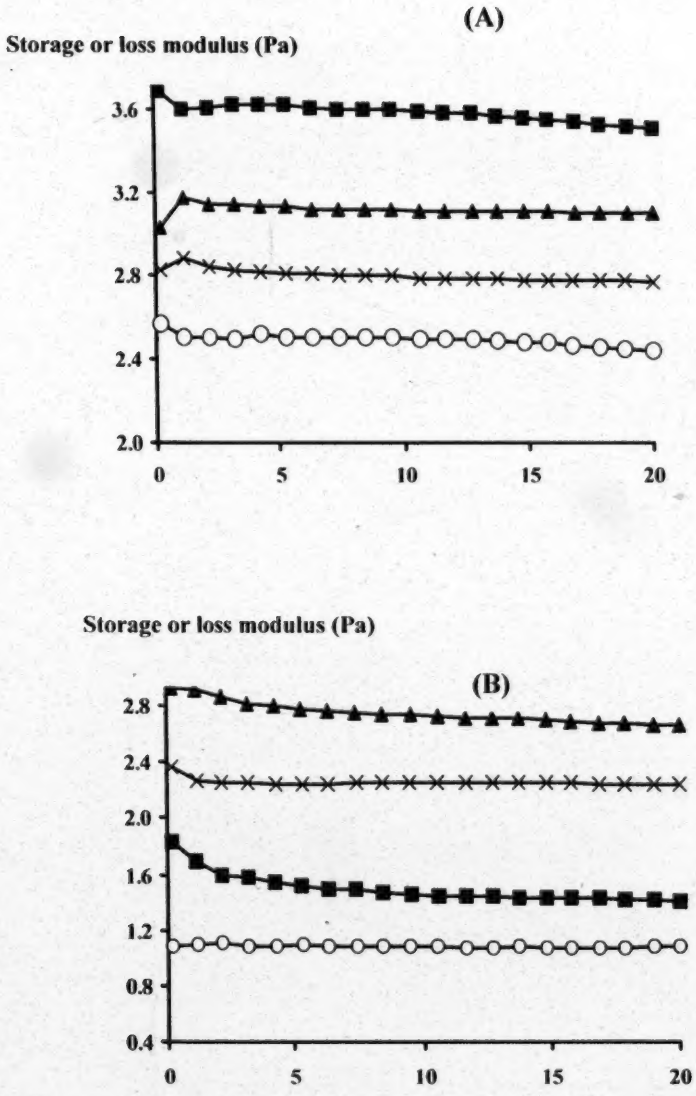
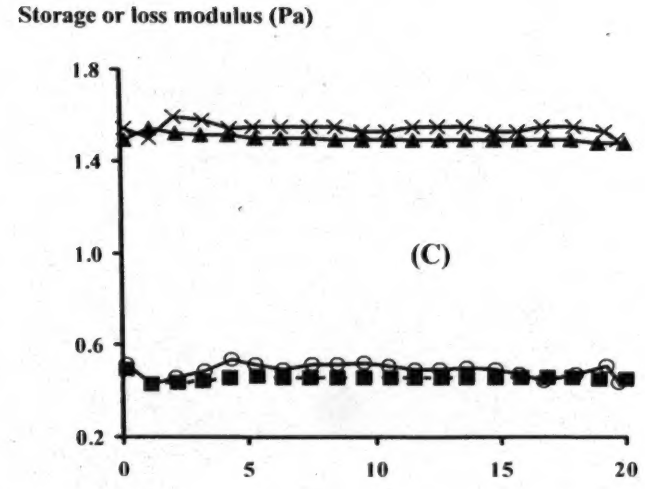


Figure 5-5 Graphs showing the storage modulus (G′) and loss modulus (G″) of 0.5% (w/w) BBG/other gum blends for (■) G′ of 80/20, w/w, (▲) G″ of 80/20, w/w, (o) G′ of 90/10, w/w, (x) G″ of 90/10, w/w.
(A) BBG/XAN, (B) BBG/CMC
(BBG= barley beta-glucan; XAN=xanthan; CMC=carboxymethyl)



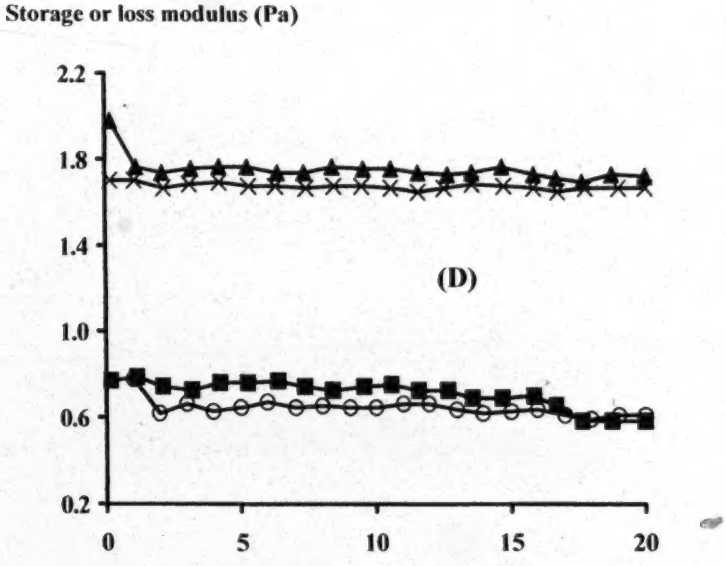


Figure 5-5 Graphs showing the storage modulus (G′) and loss modulus (G″) of 0.5% (w/w) BBG/other gum blends for (■) G′ of 80/20, w/w, (▲) G″ of 80/20, w/w, (o) G′ of 90/10, w/w, (x) G″ of 90/10, w/w.
(C) BBG/LBG, (D) BBG/GUA
(BBG= barley beta-glucan; GUA= Guar gum; LBG=locust bean gum)

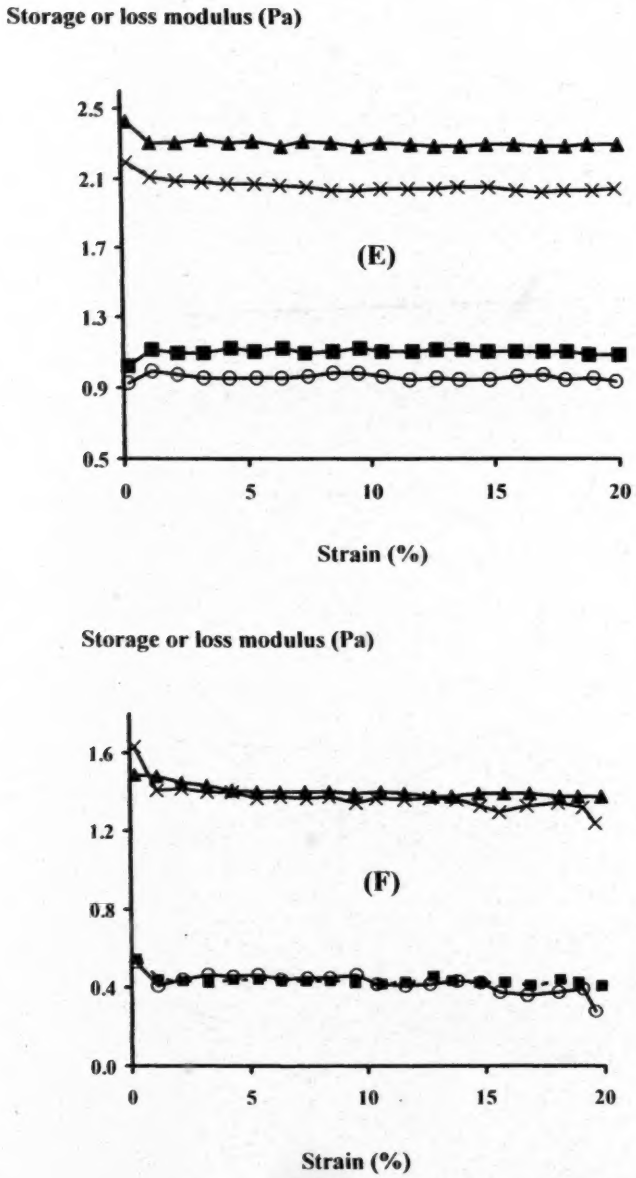


Figure 5-5 Graphs showing the storage modulus (G′) and loss modulus (G″) of 0.5% (w/w) BBG/other gum blends for (■) G′ of 80/20, w/w, (▲) G″ of 80/20, w/w, (o) G′ of 90/10, w/w, (x) G″ of 90/10, w/w.
(E) BBG/*l*-CAR, (F) BBG/KOG
(BBG= barley beta-glucan; *l*-CAR= *lambda*-carageenan; KOG=Konjac)

gum concentration of 0.5% (w/w), could not be measured for viscoelastic tests as the stress applied (1 Pa) during the amplitude sweep exceeded the strength of the network.

Figure 5-6 shows viscoelastic behavior of 0.75% (w/w) BBG/other gum blends determined at 20°C. For both gum ratios of 80/20 and 90/10 (w/w) of 0.75% (w/w) BBG/XAN blend, crossover of G′ and G″ was observed. The cross over of G′ and G″ is defined as a change from the viscoelastic fluid to viscoelastic solid (Lazaridou et al., 2003). This indicated a soft gel formation when total gum blend concentration was increased from 0.5 to 0.75%, w/w. In addition to the gum concentration, the gel setting or gelation time has been reported to be affected by time and temperature of storage (Lazaridou et al., 2003). In the present study, critical time of G′ and G″ cross over for the gum blends was not detected. Gum blends containing BBG and HMP, LMP, MCC, ALG or GAR at a total gum concentration of 0.75% (w/w) was subjected to viscoelastic tests as the stress applied (10 Pa) during the amplitude sweep exceeded the strength of the network.

5.4.5 Stability of gum blends

BBG dispersions are known to undergo phase separation when stored for a long period as BBG molecules undergo associations/aggregation via linear cellulosic segments of the molecules and precipitate (Burkus, 2001). The relative scores (as determined subjectively) for phase stability and visible precipitation for 0.5% and 0.75% (w/w) BBG/other gum blends are given in Table 5-6.

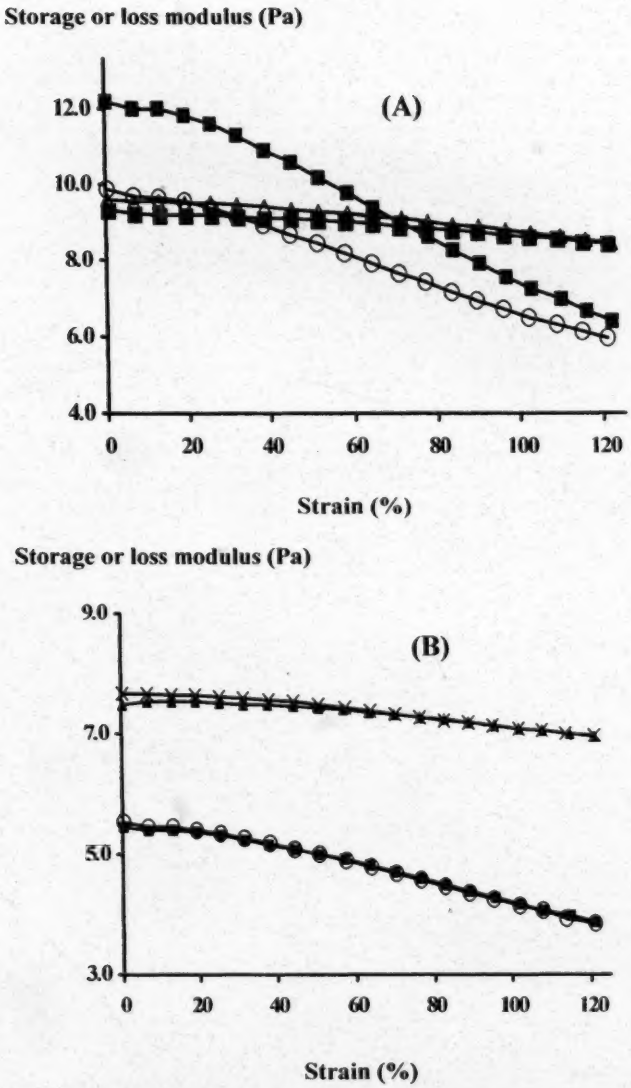


Figure 5-6 Graphs showing the storage modulus (G′) and loss modulus (G″) of 0.75% (w/w) BBG/other gum blends. (■) G′ of 80/20, w/w, (▲) G″ of 80/20, w/w, (o) G′ of 90/10, w/w, (x) G″ of 90/10, w/w.
(A) BBG/XAN, (B) BBG/CMC
(BBG= barley beta-glucan; XAN=xanthan; CMC=carboxymethyl cellulose)

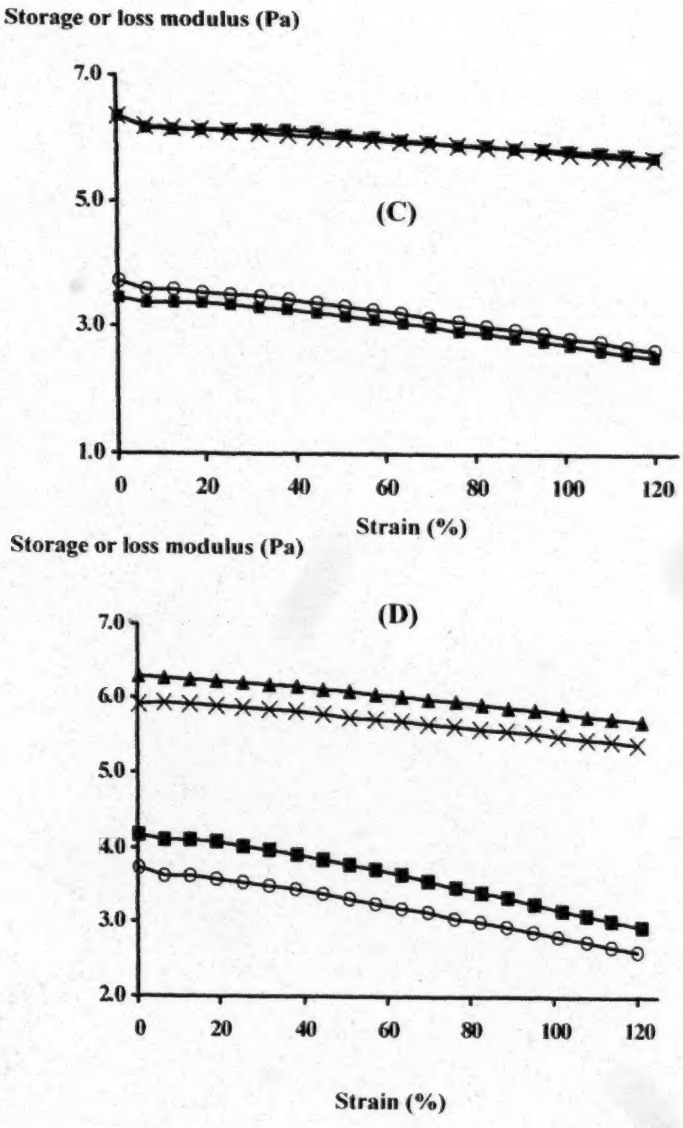


Figure 5-6 Graphs showing the storage modulus (G′) and loss modulus (G″) of 0.75% (w/w) BBG/other gum blends. (■) G′ of 80/20, w/w, (▲) G″ of 80/20, w/w, (o) G′ of 90/10, w/w, (x) G″ of 90/10, w/w.
(C) BBG/LBG, (D) BBG/GUA
(BBG= barley beta-glucan; GUA= Guar gum; LBG=locust bean gum)

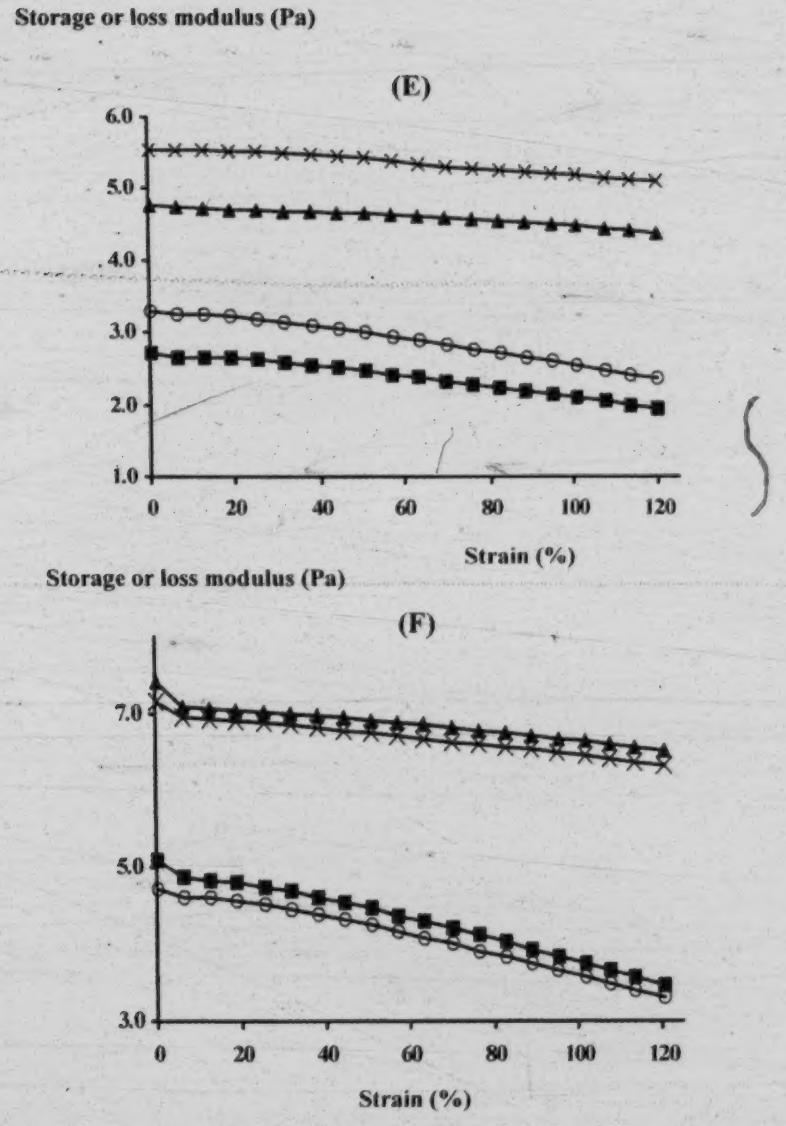


Figure 5-6 Graphs showing the storage modulus (G′) and loss modulus (G″) of 0.75% (w/w) BBG/other gum blends. (■) G′ of 80/20, w/w, (▲) G″ of 80/20, w/w, (o) G′ of 90/10, w/w, (x) G″ of 90/10, w/w.
(F) BBG/*i*-CAR, (F) BBG/*l*-CAR
(BBG= barley beta-glucan; *lambda*-carageenan; *i*-CAR=*iota*-carageenan)

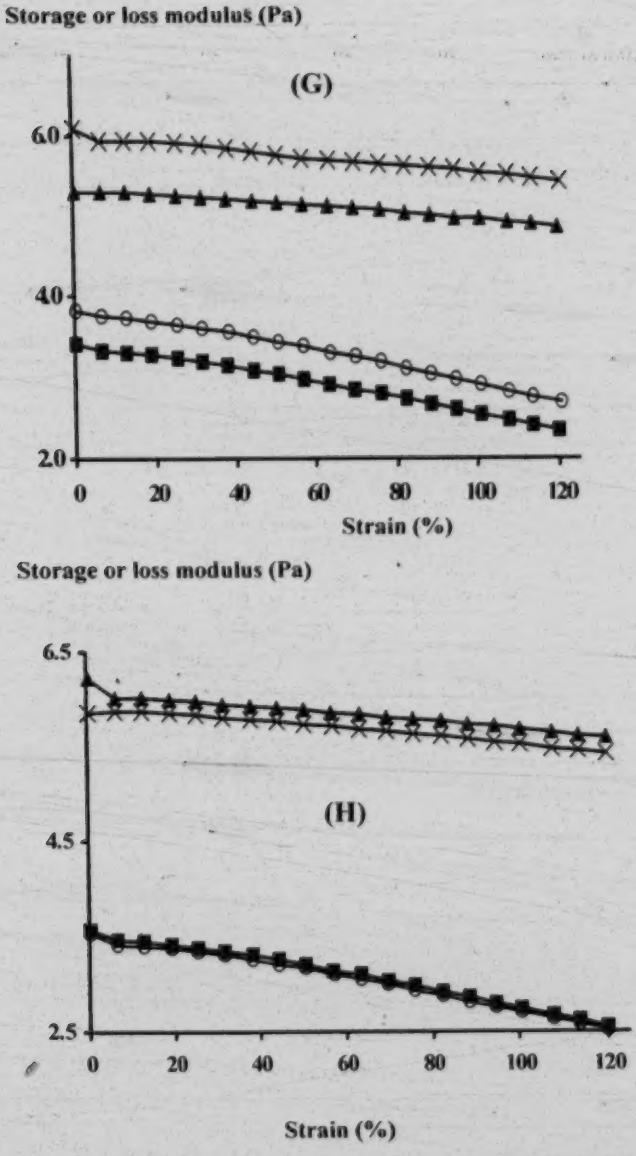


Figure 5-6 Graphs showing the storage modulus (G′) and loss modulus (G″) of 0.75% (w/w) BBG/other gum blends. (■) G′ of 80/20, w/w, (▲) G″ of 80/20, w/w, (o) G′ of 90/10, w/w, (x) G″ of 90/10, w/w.
(G) BBG/*k*-CAR, (H) BBG/KOG
(BBG= barley beta-glucan; *k*-CAR= *kappa*-carageenan; KOG=Konjac)

The phase stability of β-glucan molecules increased during the first two weeks upon increasing the total gum concentration from 0.5% - 0.75% (w/w). This is due to the increased viscosity of the dispersions at high concentration that slowed down the aggregation process of BBG molecules inhibiting the phase separation.

Unique stability properties of the BBG when blended with XAN were observed (Table 5-6). The blends were found to be stable with no signs of phase separation for more than 12 weeks of storage at ambient temperature. BBG/XAN blends having total gum concentrations of 0.5% and 0.75% (w/w) exhibited excellent phase stability against visible phase separation/precipitation due to excellent thermodynamic compatibility of gum components in aqueous medium. The mechanism behind this phenomenon may be the polysaccharide-polysaccharide complex formation. Existence of such a complex formation may be the reason behind the high degree of viscous synergism observed for these blends. Phase separation was observed for all other 0.5% and 0.75% (w/w) BBG/other gum blends. This occurred probably due to the limited thermodynamic compatibility between BBG and other gums present in the mixture.

Table 5-6 Relative stability of pure gum and gum blend dispersions at 0.5% and 0.75% (w/w) total concentration during 12-week storage at ambient temperature.

Gum blends	Gum concentration (%, w/w)	Scores[a] No. of weeks											
		1	2	3	4	5	6	7	8	9	10	11	12
BBG	0.5	1	2	2	3	3	3	4	4	4	4	4	4
	0.75	1	1	1	2	3	3	3	4	4	4	4	4
BBG/XAN	0.5	1	1	1	1	1	1	1	1	1	1	1	1
	0.75	1	1	1	1	1	1	1	1	1	1	1	1
BBG/GUA	0.5	1	3	3	4	4	4	4	4	4	4	4	4
	0.75	1	3	3	3	4	4	4	4	4	4	4	4
BBG/LBG	0.50	1	3	3	4	4	4	4	4	4	4	4	4
	0.75	1	3	3	3	4	4	4	4	4	4	4	4
BBG/HMP	0.50	2	3	4	4	4	4	4	4	4	4	4	4
	0.75	1	3	4	4	4	4	4	4	4	4	4	4
BBG/LMP	0.50	2	3	4	4	4	4	4	4	4	4	4	4
	0.75	1	3	4	4	4	4	4	4	4	4	4	4
BBG/CMC	0.50	1	1	2	3	3	4	4	4	4	4	4	4
	0.75	1	1	2	2	3	4	4	4	4	4	4	4
BBG/MCC	0.50	1	2	3	3	4	4	4	4	4	4	4	4
	0.75	1	2	2	3	4	4	4	4	4	4	4	4
BBG/ALG	0.50	1	2	3	3	4	4	4	4	4	4	4	4
	0.75	1	2	2	3	4	4	4	4	4	4	4	4
BBG/*l*-CAR	0.50	1	3	3	4	4	4	4	4	4	4	4	4
	0.75	1	3	3	3	4	4	4	4	4	4	4	4
BBG/*k*-CAR	0.50	1	3	3	4	4	4	4	4	4	4	4	4
	0.75	1	3	3	3	4	4	4	4	4	4	4	4
BBG/*i*-CAR	0.50	1	3	3	4	4	4	4	4	4	4	4	4
	0.75	1	3	3	3	4	4	4	4	4	4	4	4
BBG/KOG	0.50	2	3	4	4	4	4	4	4	4	4	4	4
	0.75	1	3	4	4	4	4	4	4	4	4	4	4
BBG/GAR	0.50	2	3	4	4	4	4	4	4	4	4	4	4
	0.75	1	2	3	4	4	4	4	4	4	4	4	4

[a] 1 - Extremely clear, no phase separation and no precipitation; 2 - clear, some phase separation and some precipitation; 3 - extreme phase separation and extreme precipitation; 4 - complete phase separation and precipitation

BBG= barley beta-glucan; XAN=xanthan; GUA= Guar gum; LBG=locust bean gum; HMP=high methoxl pectin; LMP=low methoxyl pectin; CMC=carboxymethyl cellulose; MCC=microcrystalline cellulose; ALG=alginate; *l*-CAR= *lambda*-carageenan; *k*-CAR= *kappa*-carageenan; *i*-CAR=*iota*-carageenan; KOG=Konjac; GAR= gum arabic

5.4.6 Stability of beverage formulation

Beverage samples devoid of gum demonstrated stable viscosity throughout the entire storage period (Table 5-7). The % loss of the original viscosity for pure gum solutions and gum incorporated beverage samples measured at a shear rate of 64.6 s^{-1} and at 5°C and 25°C is given in Table 5-7. The beverage samples were prepared at two concentrations, 0.23% (w/w) and 0.46% (w/w), and tested only at pH 3.25. The % loss of the original viscosity of the beverage containing BBG/XAN at 0.23% (w/w) and 0.46% (w/w) were 0.5% and 7.5%, respectively, as compared to 7% and 18.5%, respectively, for the beverage containing BBG alone. The above data clearly indicated that the incorporation of XAN is beneficial in preventing the loss of viscosity in acidic aqueous dispersions of β-glucan. This may be attributed to the high stability of XAN in acidic environments (Kovacs and Kang, 1977) and its interaction with BBG. Pure gum solutions, especially with a high gum concentration (0.46%, w/w) exhibited higher viscosity loss than 0.23% (w/w) control solutions throughout the storage period. The solution containing BBG alone (0.46%, w/w; pH 3.25) exhibited 28.5% loss of the original viscosity as compared to 17.9% loss in BBG/XAN blend (Table 5-7). Acidic condition accentuated the loss of viscosity of 0.46% (w/w) BBG dispersions as the viscosity loss progressed from 8.4% at pH 7 to 28.5% at pH 3.25. Loss in viscosity may be attributed to molecular aggregation of β-glucan via linear cellulosic segments and its precipitation out of the solution.

Table 5-7 Percentage loss[a] of original viscosity[b] (mPa.s) of pure gum solutions and gum incorporated beverage samples stored for 12 weeks at 4°C.

Percent Loss of Original Viscosity					
		pH 3.25		pH 7	
		Total concentration of gum, % (w/w)			
Type of gum or gum blend	Temperature at which viscosity determined	0.23	0.46	0.23	0.46
		Pure Gum Solutions			
BBG (control)	5°C	20.2	28.5	1.8	8.4
	25°C	20.3	32.6	1.5	7.6
BBG/XAN	5°C	12.1	17.9	4.0	11.0
	25° C	9.8	15.8	3.7	10.8
		Gum Incorporated Beverage Samples			
Beverage only (control)	5°C	0.27	0.29	nd[c]	nd
	25°C	0.50	0.61	nd	nd
Beverage + BBG	5°C	7.10	18.50	nd	nd
	25° C	9.20	25.20	nd	nd
Beverage + BBG/XAN	5°C	0.50	7.50	nd	nd
	25°C	0.60	16.80	nd	nd

Values are means of replicate determinations.
[a] Percentage loss = (loss of viscosity/original viscosity) x 100
[b] Viscosity was determined at two different temperatures, 5°C and 25°C, and at a shear rate of 64.6 s^{-1}
[c] not determined – because most beverages are acidic in nature
BBG= barley beta-glucan; XAN=xanthan

The molecular aggregation/precipitation and consequent cloud loss in BBG dispersions has been reported to be reflected by absorbance measurement at 660 nm (Bansema, 2000). Regardless of the pH, at both gum concentrations, the % loss of the absorbance (cloud loss) for pure gum dispersions containing BBG alone was substantially higher than its counterpart containing BBG/XAN blend (Table 5-8). Similarly, beverage samples containing BBG alone at both gum concentrations exhibited higher cloud loss (Table 5-8) as compared to beverage containing BBG/XAN. This is in agreement with Bansema (2000) who reported cloud loss for BBG beverages during the first three weeks of storage. Acidity negatively affected the cloud stability (increased cloud loss) of aqueous gum dispersions containing BBG alone at both 0.23% and 0.46% (w/w) total concentrations (Table 5-8).

Table 5-9 shows the relative stability (as determined subjectively/visually) of pure gum solutions and gum incorporated beverage samples during 12 weeks of storage at 4°C. Those containing 0.23% (w/w) BBG and 0.23% BBG/XAN remained as single-phase solutions for 12 weeks of storage at 4°C. This is in agreement with Bansema (2000) who reported the concentration of 0.25% (w/w) β-glucan to be lower than the phase separation threshold and therefore no phase separation. Visible precipitation in dispersions containing 0.46% BBG at both pH 3.25 and 7 was observed during the 12 week storage at 4°C. The BBG/XAN blends at total concentrations of 0.23 and 0.46% (w/w) demonstrated improved cloud

Table 5-8 Percentage loss[a] of spectrophotometric absorbance[b] as a measure of cloud stability of pure gum solutions and gum incorporated beverage samples stored for 12 weeks at 4°C

	Percent loss of absorbance values at 660 nm			
	pH 3.25		pH 7	
	Total gum concentration % (w/w)			
Type of gum or gum blend	**0.23**	**0.46**	**0.23**	**0.46**
	Pure Gum Solutions			
BBG (control)	82.7	60.8	60.2	41.5
BBG/XAN	0.33	9.7	2.5	10.8
	Gum Incorporated Beverage samples			
Beverage only (control)	1.8	1.7		
Beverage + BBG	29.3	29.5		
Beverage + BBG/XAN	2.8	5.1		

Values are means of replicate determinations.
[a] Percentage loss = (loss of absorbance/original absorbance) x 100
[b] Determined at a wavelength of 660 nm at the room temperature.
BBG= barley beta-glucan; XAN=xanthan

Table 5-9 Relative stability (as determined subjectively/visually) of pure gum solutions and gum incorporated beverage samples during 12- weeks of storage at 4° C.

Gum blends	Gum concentration (%, w/w)	Scores[a] No. of weeks 0	2	4	8	12	Comments
Pure Gum Solutions							
pH 3.25							
BBG (control)	**0.23**	1	1	3	3	4	Precipitate at bottom
	0.46	1	2	3	4	4	Precipitate at bottom
BBG/XAN	**0.23**	1	1	1	1	1	No precipitate seen
	0.46	1	1	1	1	1	No precipitate seen
pH 7							
BBG (control)	**0.23**	1	1	2	3	4	Precipitate at bottom
	0.46	1	2	3	4	4	Precipitate at bottom
BBG/XAN	**0.23**	1	1	1	1	1	No precipitate seen
	0.46	1	1	1	1	1	No precipitate seen
Gum Incorporated Beverage Samples							
Beverage only (control)		1	1	1	1	1	No precipitate seen
Beverage + BBG	**0.23**	1	1	3	3	4	Precipitate at bottom
	0.46	1	2	3	4	4	Precipitate at bottom
Beverage + BBG/XAN	**0.23**	1	1	1	1	1	No precipitate seen
	0.46	1	1	1	1	1	No precipitate seen

Values are means of replicate determinations.

[a]1 - Extremely clear, no phase separation and no precipitation; 2 - clear, some phase separation and some precipitation; 3 - extreme phase separation and extreme precipitation; 4 - complete phase separation and precipitation

BBG= barley beta-glucan; XAN=xanthan

stability with no signs of precipitation at both pH 3.25 and 7 throughout the storage period.

5.5 Conclusions

BBG in binary systems exerted synergistic interactions with XAN, *iota*-CAR, and CMC, and the interactions depended mainly on the blending ratios and the total gum concentrations. Blending of XAN into aqueous dispersions of BBG generates viscous synergism at the high total gum concentration of 0.75% (w/w) and that was not observed at the concentration of 0.5% (w/w). The improved shear tolerance (observed from flow behavior index) of BBG/XAN blends may be beneficial in food applications where enhanced shear tolerance is required. A soft gel transformation (a change from viscoelastic fluid to viscoelastic solid) when BBG was blended with XAN may provide a unique consistency needed for "solids suspension property" much desired in products such as salad dressings or other food beverages with suspended particles. The unique thermodynamic compatibility of BBG and XAN in binary gum blends as demonstrated by no phase separation observed during the 12-week storage at ambient temperature suggested its potential application in aqueous food systems. The BBG/XAN blends at neutral and acidic conditions demonstrated better viscosity stability and phase stability than those of the aqueous systems containing BBG alone. Incorporation of XAN into BBG dispersions changed the rheological properties of BBG dispersions from viscoelastic fluid to viscoelastic solid. This demonstrated the potential of BBG/XAN blends in food applications (such as salad dressings) where weak gel-like characteristics are desired. In

particular, the addition of XAN or CMC to aqueous solutions of BBG improves the shear tolerance of BG solutions meaning that at particular shear rates (eg. intestinal shear rates), blends of BBG with XAN or CMC will maintain higher viscosities than BBG alone. This finding will improve the satiety effect of BBG within the human body and may be particularly useful in the creation of diet products wishing to maintain a feeling of fullness through the use of such blends. The evidence gathered from the present study indicates the potential applications for BBG in the functional beverage industry.

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Chapter 6*
A RAPID METHOD TO QUANTITATIVELY DETERMINE THE *IN VITRO* ADSORPTION OF TAUROCHOLATE TO SOLUBLE FIBER

6.1 Introduction

Dietary fiber consists of two categories, namely soluble and insoluble fiber. Although both categories are known to confer hypocholesterolemic activity, the former is more effective than the latter (Jenkins et al., 1975; Anderson et al., 1992; Goel et al., 1998). The hypocholesterolemic activity of these fiber forms has been attributed to their viscosity, fermentability, and bile acid-binding property (Gallaher and Scheeman, 1986; Anderson, 1985; Goel et al., 1998; Kritchevsky, 1995). The subject of interest in the present study was the bile salt-adsorption capability of soluble fiber. Bowles et al. (1996) reported that the hypocholesterolemic activity of β-glucan, a soluble fiber, stems from its ability to entrap bile acids in viscous digesta rather than specific adsorption to bile acid micelles. Their method of analysis involved the use of solid state ^{13}C-nuclear magnetic resonance (NMR) of a complex between β-glucan and Congo red dye. This method is time-consuming (over 5 h), complex and requires expertise in spectroscopy. In addition, background noise is always a limiting factor in solid state NMR. Cuesta-Alonso and Gilliland (2003) used dialysis and membrane filtration to determine bile salt-adsorption to soluble fiber. Their method is simple compared to the method of Bowles et al. (1996), but nevertheless requires over three days to complete the analysis.

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A simple means to determine bile acid/salt adsorption to insoluble fiber has been developed and utilized extensively in nutrition research. The method involves mixing insoluble fiber with ^{14}C-labeled bile acids/salts, incubation at 37°C for 2 h, centrifugation and liquid scintillation counting of the supernatant. The amount of bile acid/salt bound is calculated as the difference between the amount added and that recovered in the supernatant (Goel et al., 1998). For obvious reasons, this method, as such, is not suitable for soluble fiber, but it can be modified for soluble fiber. Therefore, the main objective of this study was to develop a method to quantitatively determine the extent of bile salt adsorption to soluble fiber. Story and Lord (1987) employed a membrane filtration (ultrafiltration) technique to determine bile salt adsorption to soluble fibre. In this study, it was intended to combine the centrifugation step of the method of Goel et al. (1998) with the membrane filtration step of the method of Story and Lord (1987) into a single step in order to develop a simple method for soluble fiber.

6.2 Materials and Methods

6.2.1 Materials

Oat and barley β-glucan concentrate, concentrated forms of β-glucan (45-65%, w/w, β-glucan) were provided by Cevena Bioproducts Inc. (Edmonton, AB). Barley and oat β-glucan (BBG and OBG) in β-glucan concentrate was further purified on a laboratory scale according to Figure 6-1 through traditional aqueous extraction followed by alcohol precipitation of β-glucan in order to obtain a purified

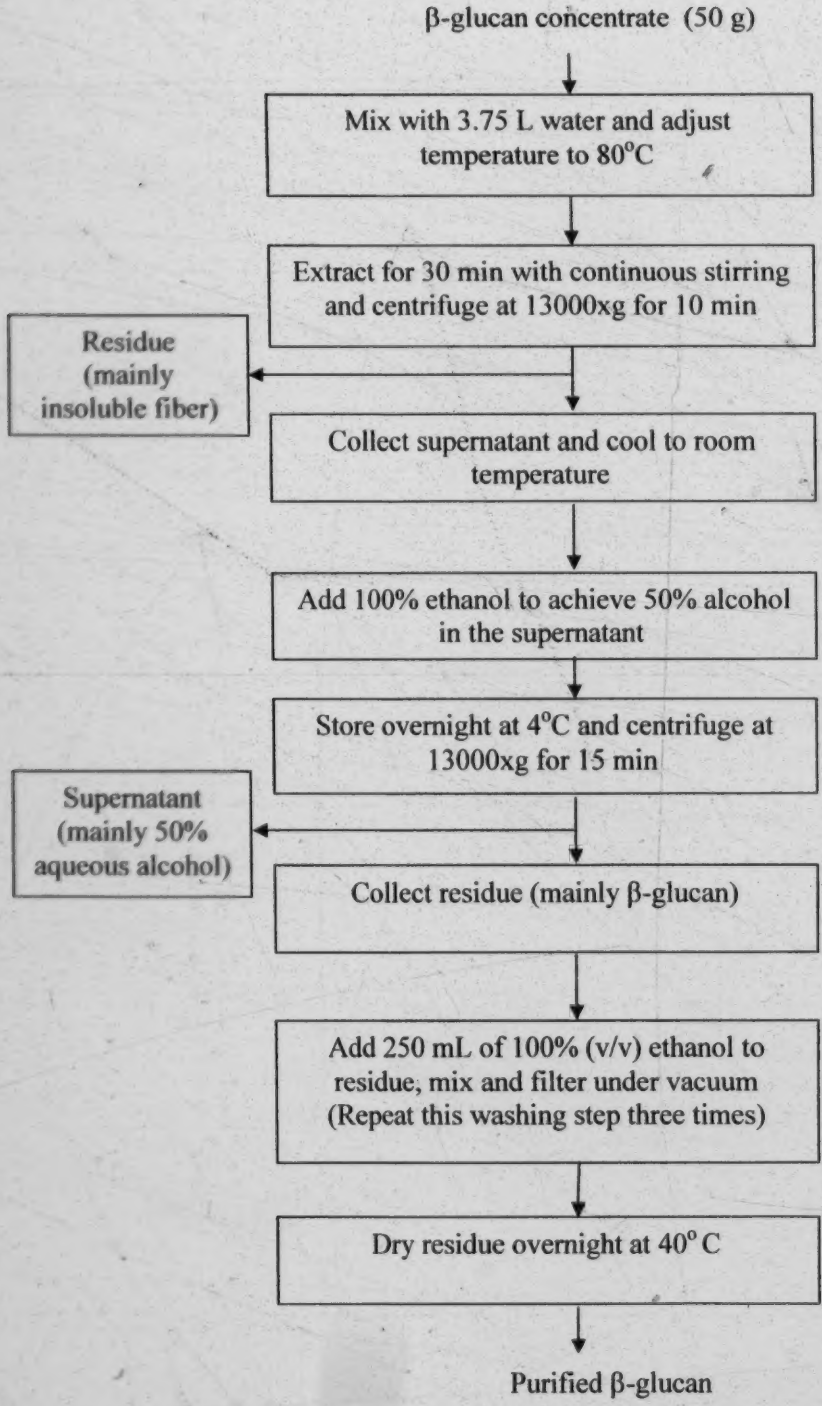
β-glucan concentrate (50 g)
Mix with 3.75 L water and adjust temperature to 80°C
Extract for 30 min with continuous stirring and centrifuge at 13000xg for 10 min
Residue (mainly insoluble fiber)
Collect supernatant and cool to room temperature
Add 100% ethanol to achieve 50% alcohol in the supernatant
Store overnight at 4°C and centrifuge at 13000xg for 15 min
Supernatant (mainly 50% aqueous alcohol)
Collect residue (mainly β-glucan)
Add 250 mL of 100% (v/v) ethanol to residue, mix and filter under vacuum (Repeat this washing step three times)
Dry residue overnight at 40° C
Purified β-glucan

Figure 6-1 Flow-chart depicting the steps involved in further purification of β-glucan from a concentrate at laboratory scale.

product containing >80% (w/w, dry basis) β-glucan. High methoxyl pectin (HMP), Low methoxyl pectin (LMP), locust bean gum (LBG), xanthan gum (XAN), guar gum (GUA), gum arabic (GAR), and carboxymethyl cellulose (CMC) were obtained from TIC Gums Inc. (Belcamp, MD). *Lamda-* and *kappa*-Carageenan (CAR), and microcrystalline cellulose (MCC) were procured from FMC BioPolymer (Philadelphia, PA). Cholestyramine resin (CHO), an antihypercholesterolemic drug, was procured from Sigma Aldrich Inc. (St Louis, MO). Cellulose (CEL) fiber was procured from Quadra Chemicals (Burlington, ON). All gum powders were of >80% (w/w) purity. Non-radioactive sodium taurocholate was obtained from MP Biomedicals, Inc. (Aurora, OH). Radioactive sodium taurocholate (specific activity of 57.0 m*Ci*/mMol) was purchased from Amersham Biosciences (Piscataway, NJ). Mono- and di-basic potassium phosphate (K_2HPO_4 and KH_2PO_4) were procured from BDH Inc. (Toronto, ON). The centrifugal filter devices (30,000 molecular weight cut off range) of 0.5 mL capacity were obtained from Millipore Corp. (Bedford, MA).

6.2.2 Preparation of radioactive bile salt (taurocholate) stock solutions

Stock solutions of unlabelled taurocholate salts were prepared in phosphate buffer (20 μM; pH 6.5) at various concentrations ranging from 5 mM – 40 mM. Appropriate amounts of radioactive taurocholate were mixed in order to ensure scintillation counts of 100,000 DPM (disintegrations per minute) per mL of the labeled solution.

6.2.3 Bile salt-adsorption assay

Soluble fiber (12.5 mg) was weighed into 2.5 mL of phosphate buffer (20 μM; pH 6.5) and heated in a boiling water bath for 45 min or until completely dispersed. Labeled bile salt (2.5 mL), prepared in different concentrations (5 mM – 40 mM), was added to the above 2.5 mL of fiber dispersion. This ensured the final mixture to contain 0.25% (w/w) fiber and 2.5 mM – 20 mM taurocholate concentration that was targeted in this assay. The mixture (5 mL) was then incubated in a shaking water bath at 37°C for 1 h. An aliquot of 50 μL was transferred into a centrifugal filter device (filter of the device was preconditioned by centrifuging 25 μL of phosphate buffer at 30,000xg for 10 min), and centrifuged for 10 min at 30,000xg. The membrane filter device was selected to have a membrane cut-off of 30,000 Daltons as all the soluble fibers included in this study possess molecular weight of more than 30,000 Daltons. The retentate on the membrane after the first centrifugation was subjected to four additional washings with 25 μL of phosphate buffer each time and centrifuged at 30,000xg for 10 min. Filtrates of all washings from the fiber samples were pooled in the same vial. Bile salt adsorption to fiber was monitored by counting the radioactivity of the pooled filtrate in a liquid scintillation counter (Beckmann Coulter Canada Inc., Missisuauga, ON). A blank devoid of soluble fiber was also run in order to account for the amount of taurocholate bound to the membrane. The level of radioactivity measured in the filtrate of the blank sample was very close to 100% of that added, indicating that there was no adsorption of bile salts to the membrane material. The amount of bile

salt adsorbed by the fiber was calculated as the difference between the amount of bile salt added and that detected in the filtrate.

6.2.4 Testing of soluble fibers/gums

OBG, BBG, and reference compounds (such as CHO and CEL) were tested for their bile adsorption capacities based on the assay as described above at a range of bile salt concentration (2.5 mM – 20 mM) for method development purposes. However, bile salt concentration was kept constant at 12.5 mM and 15 mM for testing bile salt adsorption capacities of commonly used food gums such as LBG, GUA, HMP, GAR, XAN, LMP, *k*-CAR, λ-CAR, and MCC.

6.2.5 Statistical analysis

All experiments were replicated twice. Three determinations of adsorbed bile salt concentration were performed for each replicate. Thus, all data reported are averages of 6 values. Data were analyzed using SAS general linear model (GLM) and the significance ($p \leq 0.05$) of the differences between mean values was established using Tukey's studentized range test.

6.3 Results and discussion

6.3.1 Method development

The cereal β-glucans, i.e. BBG and OBG, and reference compounds such as cholestyramine resin, an antihypercholesterolemic drug, and purified cellulose fiber were selected for method development purposes. After incubating fiber samples with labeled taurocholate, they were subjected to centrifugal filtration, the

radioactivity of the filtrate was measured and the retentate (residue) on membrane was washed repeatedly (upto six times) to ensure complete removal of free bile salt. The four washings performed on the retentate after centrifugal filtration, as shown in Figure 6-2, were sufficient to wash away any free bile salt left in the retentate as reflected by the small changes in the scintillation counts (DPM) observed in the filtrate after the fourth washing. Radioactivity of the filtrate, obtained after each washing up to third wash, decreased substantially and did not change with subsequent washings. Repeated washing of the residue is a critical step of the assay because incomplete removal of free bile salt can contribute to overestimation of adsorption levels for the test fiber. Also important is to account for the bile salt interacting with the membrane of the filter device. The membrane used in this study had a very low affinity to bile salts and had large enough pores, which did not clog upon filtration.

The bile salt adsorption capacities of all the samples were performed at sodium taurocholate concentrations of (2.5 mM - 20 mM). Different taurocholate concentrations have been shown to affect bile salt adsorption capacities of the soluble dietary fibers (Cuesto-Alonso and Gilliland, 2003). As shown in Figure 6-3, the bile salt adsorption increases as the taurocholate concentration was increased from 2.5 mM to 20 mM. The amount of bile salt adsorbed to the samples (Fig. 6-3) reached a plateau at a taurocholate concentration of 12.5 mM or 15mM after which no further change in the bile salt adsorption was observed. At 12.5 mM taurocholate concentration BBG demonstrated bile salt adsorption of ~0.87 μmol/mg of fiber

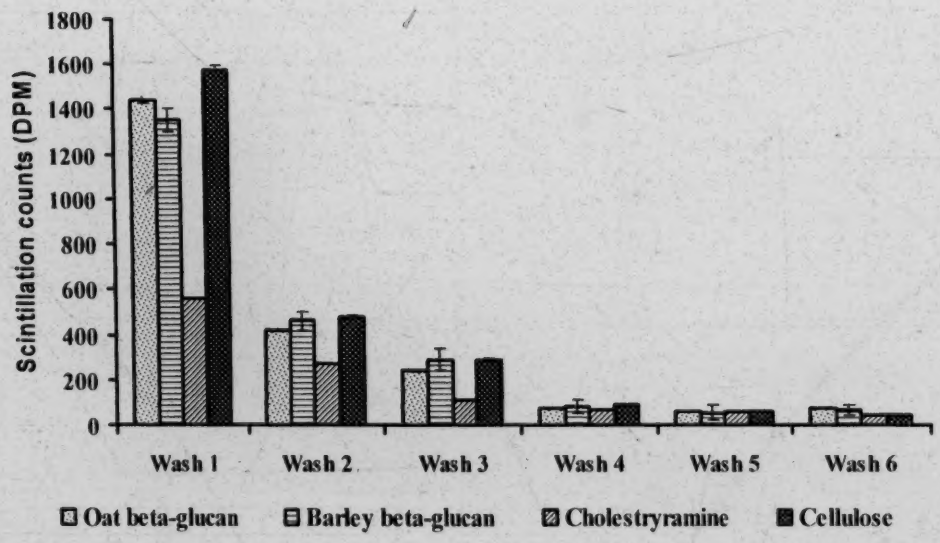

Figure 6-2 Scintillation counts (measured as, disintegrations per minute, DPM) of the filtrate collected separately during each successive washing of the retentate following incubation of ^{14}C-taurocholate with barley β-glucan (BBG), oat β-glucan (OBG), cholestyramine resin (CHO) and cellulose (CEL).

while bile salt adsorbed to cholestryramine was ~2.68 μmol/mg of resin. This is in agreement with the data reported for cholestyramine resin by Lee et al. (2002). Cellulose showed the least bile adsorption among all the samples evaluated in the present study. The bile salt adsorption of both types of cereal β-glucans, OBG and BBG, were similar. As shown in Figure 6-3, a taurocholate concentration higher than 15 mM demonstrated that saturation level was reached in bile salt adsorption capacity by the soluble fibers. This is in agreement with the data showing the saturation at high taurocholate concentrations to the soluble fibers (Story and Lord, 1987; Cuesto-Alonso and Gilliland, 2003).

6.3.2 Bile salt-adsorption efficacy of selected food gums

Employing the newly developed bile adsorption assay, several commercial food gums with varying molecular structures were assayed. The bile salt adsorption efficacies of all the soluble fibers were compared to that of cholestyramine resin. For all experiments coefficient of variation (CV) of <5% was achieved indicating high level of reproducibility of the data. Figures 6-4 and 6-5 depict the amount of bile salt bound to the food gums at 12.5 mM and 15 mM taurocholate concentrations, respectively. Purified BBG and OBG were found to be similar ($p>0.05$) to each other but they showed significantly higher ($p\leq0.5$) bile salt adsorption than any of the other fibers at both 12.5 mM and 15 mM salt concentrations. At both 12.5 and 15 mM taurocholate concentration, GUA showed appreciable adsorption but was significantly lower than OBG and BBG ($p\leq0.05$) (Fig. 6-4 and 6-5). This is not in agreement with the data reported by Cuesta-Alonso and Gilliland (2003). In their

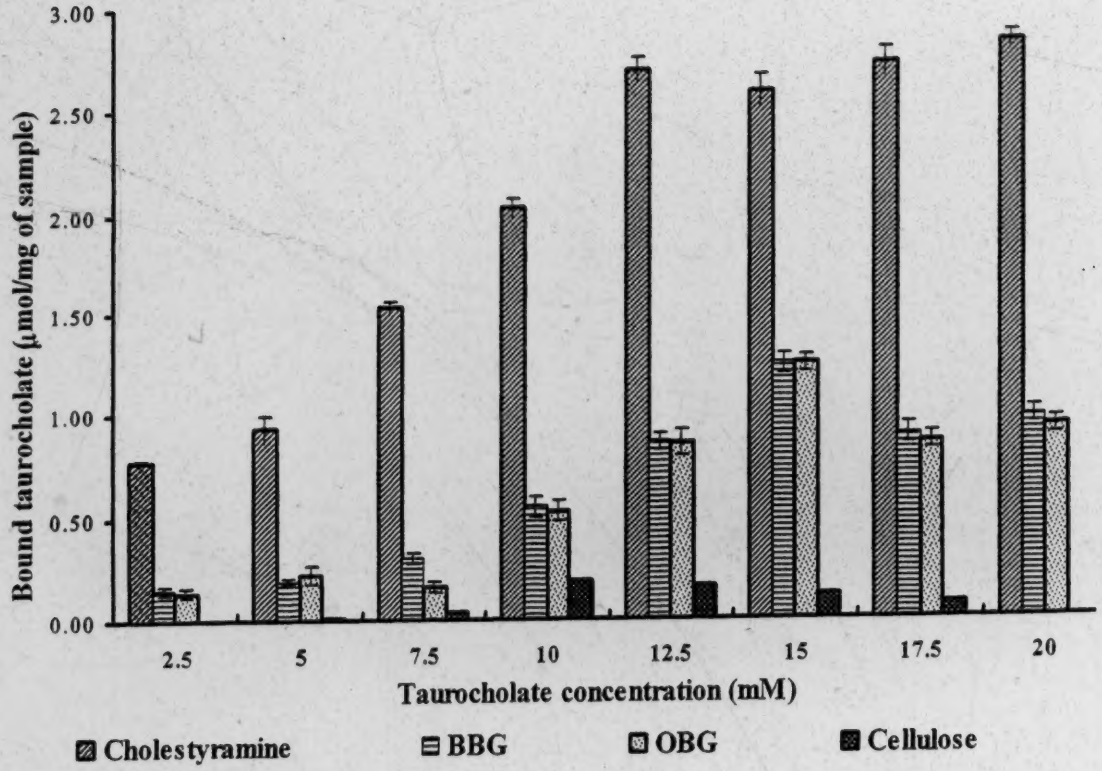

Figure 6-3 Effect of sodium taurocholate concentration (2.5 mM - 20 mM) on taurocholate adsorption of 0.25% (w/w) aqueous dispersion of barley β-glucan (BBG), oat β-glucan (OBG), cholestyramine resin (CHO) and cellulose (CEL).

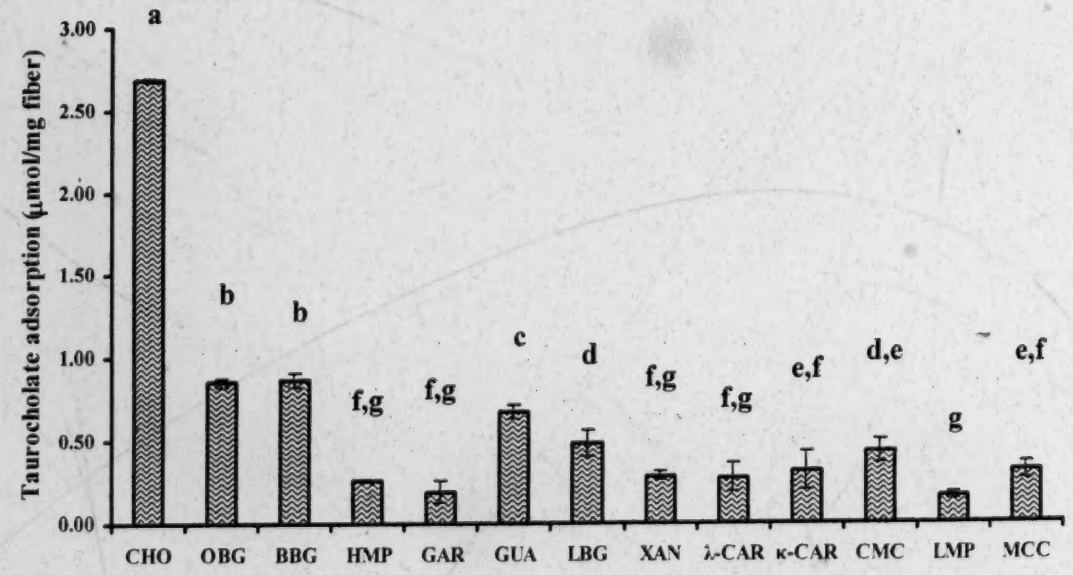

Figure 6-4 Adsorption of taurocholate (µmol/mg of fibre) by various commercially used soluble fibers/gums* evaluated at 12.5 mM sodium taurocholate concentration.

(Bars with the same letter are not significantly different ($p>0.05$). [*cholestyramine resin (CHO); oat β-glucan (OBG); barley β-glucan (BBG); High methoxyl pectin (HMP); cellulose (CEL);; gum arabic (GAR); guar (GUA); locust bean gum (LBG); xanthan (XAN); *Lamda*-Carageenan *(l-CAR); kappa*-Carageenan *(k-CAR)*; carboxymethyl cellulose (CMC); Low methoxyl pectin (LMP) and microcrystalline cellulose (MCC)]

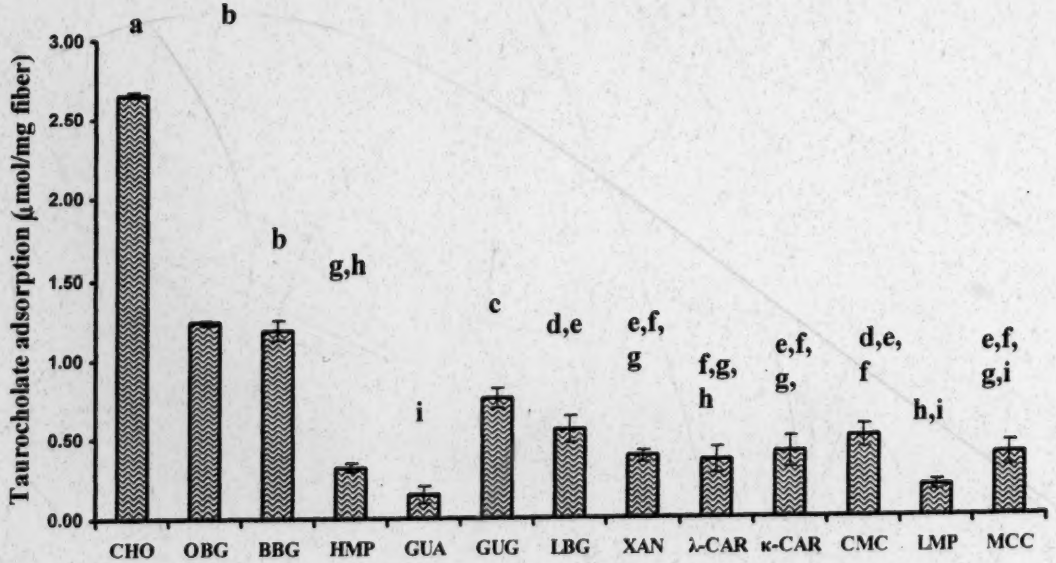

Figure 6-5 Adsorption of taurocholate (µmol/mg of fiber) by various commercially used soluble fibers/gums* evaluated at 15 mM sodium taurocholate concentration.

(Bars with the same letter are not significantly different ($p>0.05$). [*cholestyramine resin (CHO); oat β-glucan (OBG); barley β-glucan (BBG); High methoxyl pectin (HMP); cellulose (CEL);; gum arabic (GAR); guar (GUA); locust bean gum (LBG); xanthan (XAN); *Lamda*-Carageenan *(l-CAR); kappa*-Carageenan *(k-CAR)*; carboxymethyl cellulose (CMC); Low methoxyl pectin (LMP) and microcrystalline cellulose (MCC)]

study, GUA showed higher taurocholate adsorption compared to OBG. Other gums, HMP, GAR, XAN, LMP, *k*-CAR, MCC and λ-CAR demonstrated lower sodium taurocholate adsorption ($p \leq 0.05$) than BBG, OBG, GUA. LBG and CMC studied at both 12.5 mM and 15 mM taurocholate concentrations. Although the mechanism of bile salt adsorption to these gums cannot be deduced from this assay, the assay's ability to distinguish the differences in bile salt-adsorption capacities among the gums used in this study is clearly demonstrated. In recent literature, the mechanism of bile salt adsorption to BBG and OBG, which lack positively charged groups, has been linked to their viscosity (Gallaher and Scheeman, 1986; Goel et al., 1998). GUA and LBG having a galactomannan structure also lack positively charged sites and hence viscosity might have played a role in their adsorption of bile salts as well. In the present study, galactomannans (LBG and GUA) bound almost twice as much of the bile salt as HMP and three times as much as LMP at both 12.5 and 15 mM taurocholate concentrations. Viscosity, polysaccharide structure, and ionic characteristics have been indicated elsewhere to be the principal factors contributing to bile acid adsorption (Potty, 1996; Lee at al., 2002; Yamamoto et al., 2000). Xanthan (XAN) dispersions had been reported to have 5-6 fold higher viscosity as compared to that of OBG and BBG dispersions (refer to Chapter 5), but bile salt adsorption capacities were almost one-third of those demonstrated by BBG and OBG dispersions. These results demonstrate that the viscosity of dispersions may not be the only factor responsible for bile salt adsorption capability. HMP, LMP and XAN carry negatively charged groups that can effectively hinder the entrapment of

negatively charged taurocholate in the viscous polymer dispersion, resulting in low adsorption. The degree of methoxylation of pectin was not found to influence bile salt adsorption characteristics. Carboxymethyl cellulose (CMC) and LBG demonstrated similar levels of bile salt adsorption at both 12.5 mM and 15 mM taurocholate concentrations. *Kappa-* and *lambda*-carageenan differing only in their degree of sulfonate esters (~24 and ~27%, respectively) demonstrated similar bile salt adsorption capacities ($p \geq 0.05$) at both 12.5 mM and 15 mM taurocholate concentrations.

6.4 Conclusions

Membrane filtration coupled with centrifugation is a convenient means of quantitatively determining the extent of bile salt adsorption to β-glucan and other soluble fibers. A coefficient of variation (CV) of <5% indicates that the data are highly reproducible. Among the food gums evaluated, BBG, OBG and GUA have shown superior taurocholate adsorption as compared to LBG, CMC, HMP, LMP, XAN, GAR, MCC, *λ*-CAR, and *k*-CAR.

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Chapter 7
CONCLUSIONS AND RECOMENDATIONS

Understanding and evaluating product structure-function relationships offer the fundamental basis for developing and evaluating new food processing technologies that emphasize product quality while maintaining process cost efficiency. This thesis represents the evaluation of β-glucan concentrate that is produced by a novel process in a more efficient manner than is commercially available, without sacrificing product quality. The research is reported in four chapters (Chapters 3 to 6), where primary investigation into β-glucan structure led to research on its functional properties. The first investigation focused on fine structural details of β-glucan purified from a fiber concentrate manufactured by a novel patented technology (Vasanthan and Temelli, 2002). In Chapter 3 the question was asked: "Has the new concentration process preserved the native structure of β–glucan?". Structural analysis demonstrated that β-glucan's structural integrity was not altered during processing.

These results stimulated further investigation of β-glucan structure in order to lay the foundation for understanding physical functional properties (eg. solubility, rheology, etc). The preliminary proximate investigation showed phosphorous is present in both purified oat and barley β–glucan from concentrates (0.201 and 0.092%, w/w, respectively). The associations of phospholipids, phosphoproteins and esterified phosphates (phosphate monoesters) with native starch have been

demonstrated to affect the water-binding capacity, solubility, swelling and clarity of starch paste. The related question was asked: "Could β-glucan, a close structural analog of starch, have similar associations with phosphates?". It was realized that elucidation of this potential association would give significant insight regarding β-glucan solubility, which until now had been considered to be a neutral compound. The findings of Chapter 4 clearly demonstrated that β-glucan was associated with phosphate moieties in both oat and barley concentrates (DS = 0.011 and 0.005, respectively). Although the effect of phosphate substitutions on the solubility of β-glucan was not evaluated in this research, it was the first time that an association between phosphates and β-glucan has been proven (Chapter 4). Chapters 3 and 4 established a foundation to justify further work to understand β-glucan solubility with respect to phosphorus content, a project that was beyond the scope of this thesis.

Viscosity is the essential property of β-glucan fiber concentrate conferring textural attributes and health benefits. Having established that structural integrity remained intact during processing, viscosity was the obvious first functional characteristic to investigate in the fiber concentrate. In preliminary investigations, the new β-glucan concentrate manufactured by new technology had superior viscosity characteristics compared to commercially available β-glucan concentrates. This finding was consistent with the finding that the new concentrate had a higher average molecular weight compared to concentrates generated by other methods (unpublished results). Having established the excellent viscosity characteristics of

the fiber concentrate, a poorly studied yet important functional research question was addressed next, "How does β-glucan interact with the most commonly used thickeners?" The motivation behind this question was two-fold. On one hand, there is a cost incentive to reduce and optimize the use of expensive ingredients like β-glucan by understanding possible interactions with other less costly thickeners such as gums. On the other hand, many novel functional properties for new food product development are discovered by exploring combinations and interactions, such as the well-documented synergistic interaction between *kappa*-carageenan and locust bean gum (Tako et al., 1998). In Chapter 5, viscosity, thixotropy and elastic modulus parameters were measured for β-glucan and twelve common food gums in combination and alone, as binary systems. Viscosity synergism was observed for β-glucan in combination with xanthan (not observed at 0.5% total blend concentration), *iota*-carageenan, and carboxymethyl cellulose. The xanthan/β-glucan mixtures (90:10 and 80:10 by weight, respectively) was clearly superior to other mixtures and demonstrated novel functional properties such as higher viscosity and soft-gel transformation. Not only did it show that ingredient cost savings by viscosity synergism can be achieved, but also it demonstrated the ability to enhance β-glucan shear tolerance.

These experiments inspired other questions: "Would these combinations that are able to promote viscosity synergistically also be able to improve the stability of β-glucan's viscosity in aqueous systems during its shelf life?". It was hypothesized that the demonstrated indications of viscosity synergism, shear tolerance and gel

formation with the combination of β-glucan and xanthan would also predict an improvement in the stability during storage. As expected, only the xanthan/β-glucan combinations possessed unique phase stability for over 12 weeks compared to less than 1 week for β-glucan alone (Chapter 5). Also, at acidic conditions commonly found in fruit beverages (pH 3.25), xanthan/β-glucan combinations showed less viscosity degradation than β-glucan alone during storage over 12 weeks. This remarkable finding shows for the first time that β-glucan can be used in food applications as a stable thickener when combined with other thickeners. Chapter 5 described pioneering studies that could pave the way to a new branch of research to understand potential functional applications for β-glucan as a food ingredient.

In the current market, it is not enough that a processed food meets a set of desired physical qualities. As always, researchers and manufacturers of processed foods must consider basic physical attributes while providing a pleasing and good tasting product. However, in addition to fulfilling basic nutritional requirements, processed food product development is now emphasizing health benefits that extend beyond the expectation of even whole food diets. To meet recent nutritional trends and market demands, the question was asked, "From a physiological perspective, does the new β-glucan fiber concentrate perform better than commonly used soluble dietary fibers?". To answer this question, bile acid adsorption was investigated since it is the most important physiological mechanism attributed to cholesterol-lowering functionality of soluble dietary fiber. Chapter 6 demonstrated that β-glucan could adsorb bile acids better than any of the twelve gums investigation. The significance

of this finding is that it links the new fiber concentrate to a large amount of literature giving evidence for health benefits attributed to β-glucan by this mechanism (refer to Chapter 2). Currently, the Grain Science Laboratory at the University of Alberta is further investigating the effects of β-glucan and gum combinations on bile acid adsorption. Other health benefit mechanisms could be investigated in the future such as swelling-induced satiety, prebiotic functionality (colonic pH, short chain fatty acids, etc.), immune system modulation, and glucose adsorption in the context of diabetes management. It should be noted that current methodologies for measuring bile acid adsorption by soluble fibers were cumbersome; they were either time consuming (more than 3 days) or required complex technical expertise. A new quantitative method for determining bile acid adsorption by soluble fibers was adapted (Chapter 6) to facilitate screening-type development research. This new technique was born out of necessity to have a high throughput, reliable, and accurate screening process capable of analyzing many samples in one work day.

In a broader sense, the research achieved in this thesis goes beyond β-glucan processing. The research strategy examplifies a rational screening method that can systematically be applied to any new food processing technology in the food industry. In new process investigation, emphasis should always be given to a thorough and relevant understanding of the product from micro- and chemical structural perspectives. Structure predicts function, so initial structural investigation can verify the preservation of structural and chemical properties, and further research can then be warranted for evaluating and testing functional properties from both

physical and physiological perspectives. Furthermore, an understanding of fine structure and chemistry provide the underlying basis for predicting and controlling physical functionality in new food formulations or food supplements, with the potential for improvement, not just maintenance, of quality. It is a strategy reflecting the knowledge that I have gained over the course of my doctoral studies that includes not only to evaluate a new food processing technology but also to progressing in a logical sequence from a study of chemical structure to physical functionality and finally to physiological functionality.

7.1 References

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